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Rīga Stradiņš University

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Association of Epithelial Mesenchymal Transition, Stemness and Inflammation in Colorectal Carcinoma

Summary of the Doctoral Thesis for obtaining a doctoral degree "Doctor of Science (*PhD*)"

Sector – Basic Sciences of Medicine, including Pharmacy
Sub-Sector – Pathology

Riga, 2022

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Abbreviations used in the Thesis

AAM	alternately activated macrophages
Ag	antigen
CD	cluster of differentiation
CI	confidence interval
CLR	Crohn's disease like lymphoid reaction
CRC	colorectal cancer
CRP	C reactive protein
CSC	cancer stem cells
DNA	deoxyribonucleic acid
EMT	epithelial-mesenchymal transition
HE	haematoxylin and eosin
HPF	high power field
IHC	immunohistochemistry
IQR	interquartile range
IT	intratumourous
LN	lymph node
MLH1	mutL homolog 1 gene / protein
MMAH	monoclonal mouse antibody against human antigen
MMR	mismatch repair gene / proteins
MRAH	monoclonal rabbit antibody against human antigen
MSH2	MutS homolog 2 protein
MSH6	MutS homolog 6 protein
MSI	microsatellite instability
MSS	microsatellite stabile
OM	original magnification
pG1-4	characteristics of tumour differentiation

pM0-1	designation of the presence or absence of distant metastasis in a patient affected by malignant tumour by pathology examination
PMS2	PMS1 homolog 2
pN1-2	characteristics of regional lymph node status regarding metastases of malignant tumour by pathology examination
PT	peritumourous
pT1-4	characteristics of tumour local spread by pathology examination
pTis	in situ carcinoma
sCRC	synchronous colorectal carcinoma
SD	standart deviation
TAM	tumour associated macrophages
TNM	tumour, node, metastasis: classification of malignat tumour by the anatomic disease extent reflecting the local spread of tumour (T), status of regional lymph nodes regarding tumour metastases (N) and presence of distant metastasis (M)
USA	United States of America

Introduction

Colorectal cancer (CRC) has been the focus of myriad scientific studies, and it remains a hot research topic in oncology. Nevertheless, CRC still represents one of deadliest tumours worldwide. According to global cancer statistics (GLOBOCAN), 19.3 million new CRC cases were diagnosed and 0.94 million CRC-related deaths occurred in 2020 (Xi & Xu, 2021).

One of the factors affecting CRC carcinogenesis and progression is inflammation. In an already established tumour, an inflammatory reaction can either promote or suppress tumour progression. Inflammatory cells are able to produce growth factors that stimulate the proliferation of neoplastic cells, to enhance angiogenesis or to degrade the connective tissue matrix that in turn facilitates invasion. Consequently, inflammation might create a microenvironment that is beneficial for tumour development. On the contrary, inflammation can induce cancer cell death (Galdiero, Garlanda, Jaillon, Marone, & Mantovani, 2013; Hanahan & Coussens, 2012). One of the main factors involved in tumour progression is the pro-inflammatory cytokine tumour necrosis factor α (TNFα), produced by macrophages. TNFα also has a crucial role in epithelial-mesenchymal transition (EMT) as it induces overexpression of the transcription factor (TF) Snail, leading to downregulation of E-cadherin and upregulation of N-cadherin (Wang et al., 2013; Z. Zhang et al., 2019), which are among the key molecules involved in EMT. Another key molecule involved with EMT is β-catenin; its expression is also known to be affected by inflammatory cytokines and to affect the development of CRC (Keerthivasan et al., 2014). Stem cell differentiation by CD44 expression in CRC (Mukohyama, Shimono, Minami, Kakeji, & Suzuki, 2017) is strongly associated with EMT (Fabregat, Malfettone, & Soukupova, 2016) and currently represents an attractive treatment target (Xu et al., 2019). However,

the relationship between CD44, E-cadherin, N-cadherin, and β-catenin expression, and local inflammation has not been studied extensively.

The response to treatment can depend on the degree of tumour heterogeneity. It is described not only in CRC (Molinari et al., 2018; Zlatian et al., 2015), but also in other carcinomas (Prince et al., 2007). Regarding CRC, the heterogeneity is more frequent in tumours exhibiting microsatellite instability (MSI) than in microsatellite-stable (MSS) tumours (De Smedt et al., 2015; McCarthy et al., 2019). Changes in mismatch repair (MMR) protein expression detected via immunohistochemistry (IHC) could be a hallmark for further DNA sequencing and personalized treatment choice.

Some studies have revealed links between decreased inflammation and the development of EMT-related tissue changes (Witschen et al., 2020). However, the results are controversial. Considering these prognostic trends, associations between the listed factors might be hypothesized, but few researchers have tried to directly assess the mutual relationships between inflammation, EMT, stem cell differentiation, and the expression of MMR proteins in the same cohort of CRC cases.

The **aim** of this work was to investigate the association between peritumoural inflammation and known adverse morphological features of CRC, including local spread (pT), the involvement of regional lymph nodes (pN), and manifestations of invasive growth as well as with the molecular landscape of EMT, cancer stem cell (CSC) differentiation, and expression of MMR proteins in Latvian patients. To achieve this aim, the following tasks were conducted:

1. Characterize demographic data, and gross and microscopic morphological features of CRC by pTNM, grade, manifestations of invasive growth, synchronous colorectal carcinoma (sCRC), and tumour volume.

2. Assess the molecular profile of CRC by immunohistochemical evaluation, analyzing the expression intensity of markers showing mesenchymal and stem cell differentiation in CRC.
3. Evaluate peritumoural inflammation and Crohn's-like inflammatory reaction (CLR), as well immunohistochemical CD8+ T cell presence within CRC.
4. Evaluate MMR protein expression within CRC tissue by using IHC.
5. Analyse the interrelations of the clinical, morphological, and molecular parameters.

Hypotheses of the Thesis

1. Local inflammation shows a significant association with CRC progression.
2. The DNA MMR proteins MSH2, MSH6, MLH1 and PMS2 have a role in epithelial mesenchymal process.

Novelty of the Thesis

1. Immunohistochemical CD44 expression in study group in difference from cell culture studies does not show correlation to inflammation degree;
2. This study is one of the first complex studies, that describe associations between tumour stemness and inflammation in human colorectal carcinoma tissue, and points out immune cell, specifically, eosinophil leucocyte possible role in tumour protection;
3. Complex approach in tissue studies reveals significant associations in difference from linear cell culture studies.

Author contribution

The author has performed all stages of the study, including the study design, selection of IHC markers, evaluation of IHC results, measurements, and statistical data analysis. The author also performed the IHC visualization and took all the gross and microscopical photographs presented in this work.

Ethical concerns

The research was carried out in accordance with the Declaration of Helsinki and received approval from the Committee of Ethics of Rīga Stradiņš University [No E-9 (2), 04.09.2014].

1 Materials and methods

1.1 The study model and ethical principle

The study was designed as a retrospective morphological and immunohistochemical investigation of a representative group of consecutive, surgically treated CRC cases. It was carried out in accordance with the Declaration of Helsinki, and it received approval from the Committee of Ethics of Riga Stradins University (No E-9 (2), 04.09.2014).

1.2 Patient identification and cohort selection

The study included 553 consecutive, potentially radically operated CRC cases, which were identified by archive search in a single university hospital from January 2011 to December 2014. The inclusion criteria were potentially radical surgery and diagnosis of CRC (adenocarcinoma, mucinous cancer, medullary cancer, undifferentiated cancer, and signet ring cell cancer). Patients who had a colorectal tumour of a different histogenesis (neuroendocrine tumour, squamous cell cancer, etc.) or other tumour metastasis, as well as those patients who did not undergo potentially radical surgery (had a biopsy) or did not have an invasive tumour (pTis), were excluded from this study.

Demographic information, such as age at the time of cancer diagnosis, gender, the presence of perforation, synchronous CRC presence, the type of operation, and the operated side, were collected from the medical histories and surgical reports, submitted to the Department of Pathology by Department of Surgery.

The location of the tumour within the bowel and the type of surgery were verified with the surgical reports, obtained from the Department of Surgery. Morphological data about tumour grossing (appearance, size) were obtained

from pathological reports. Microscopic re-assessment was done for to verify the diagnosis and to perform measurements.

1.3 Gross examination data

The gross examination data included the data about submitted organs, tumour and the lymph nodes. It proceeded based on the following scheme.

1. First, surgical material was described and measured in three dimensions for each of submitted organs;
2. Second, the tumour(s) was (were) evaluated and described in detail, including:
    - distance from the closest resection margin;
    - tumour size in three dimensions;
    - assessment of tumour appearance (circular vs. half-circular; bowl shaped vs. ulcer; mushroom or node-like).
3. Third, lymph nodes were assessed in mesenterial fat tissue; if no lymph nodes were grossly detected, mesenterial fat tissue was collected for further investigation.

The gross examination data served as the basis for further evaluation of the tumour, including tumour volume, origin, and assessment of pTN, as well as for identification of the tumour localization within bowel.

1.4 Tissue processing and microscopic examination

The tissue samples that were dissected during grossing were fixed in 10 % neutral-buffered formalin (Sigma-Aldrich, Saint Louis, MO, UA) and processed by increasing grades of 2-propanol (Sigma-Aldrich), followed by incubation in histograde xylene (J. T. Baker, Deventer, the Netherlands) and paraplast (Diapath S.r.l., Belgamo, Italy) in the Tissue-Tek® VIP™ 5 vacuum infiltration

processor (Sakura Seiki Co., Ltd., Nagano, Japan). The processed tissue was then embedded in paraplast (Diapath S.r.l.) using the TES 99 tissue embedding system (Medite GmbH, Burgdorf, Germany). After embedding, 4 μm sections were cut from the paraffin blocks with a Microm HM 360 microtome (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and placed on glass slides (Menzel-Glaser, Braunschweig, Germany). The slides were stained with haematoxylin and eosin (HE) by using a TST 44 automated tissue stainer (Medite Medizintechnik, Burgdorf, Germany) and covered with cover glass (Biosigma, Cona, Italy) using an automated cover slipper (Dako, Glostrup, Denmark) and Pertex covering medium (Histolab, Gothenburg, Sweden).

Standard slides, stained by haematoxylin and eosin, were examined under a light microscope to obtain data about histological type of primary tumour and synchronous tumours, tumour characteristics by pTNM classification (pT for local tumour invasion: T1–T4; pN for LN metastasis: N0, N1a, N1b, N1c, N2, Nx; pM for distant metastasis: M0, M1, if applicable), tumour biological potential by differentiation grade, intravascular (separately venous and arterial) invasion, peri- and intra-neural invasion, lymphatic vessel invasion, tumour necrosis, stromal desmoplasia, peritumourous inflammation reaction and Crohn's lymphoid- like inflammation in a tumour.

The peri- and intraneural invasion as well as intravascular (venous and arterial) and lymphatic vessel invasion were assessed as a categorical variable: present or absent. The spread of tumour necrosis was measured as the relative area of tumour occupied by necrosis in all tumour slides, as demonstrated by (Vayrynen et al., 2016). Additional presence of necrosis was categorized into three groups: focal, moderate, or extensive.

Tumour volume was assessed using ellipsoid formula (Tirumani et al., 2016).

Peritumoural inflammation was assessed according to the Klintrup-Mäkinnen inflammation score, by identifying four groups: no inflammation, mild inflammation, moderate inflammation, and severe inflammations. They were then distributed into two classes: low-grade (no or mild inflammation) versus high-grade (moderate or severe) inflammation (Klintrup et al., 2005). CLR was assessed according to the Vayrynen criteria, by counting CLR follicles in the invasive front, and it was defined as CLR density (Väyrynen et al., 2013).

The complete scheme of microscopical investigation and evaluated parameters in this study is depicted in Figure 1.1.

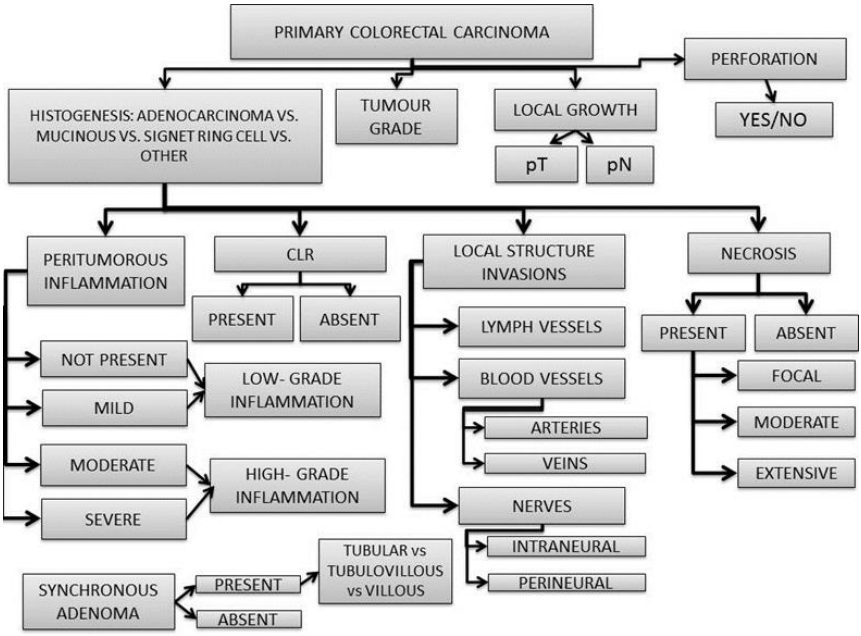

Figure 1.1 **Parameters in a microscopical evaluation in a study group**

Prepared by Inese Briede.

1.5 Immunohistochemical visualisation and assessment

Immunohistochemical visualization of the researched antigens was performed on formalin-fixed paraffin-embedded CRC and non-neoplastic control tissues. For IHC, 124 consecutive cases were selected. From each case, a representative block of formalin-fixed paraffin-embedded tumour tissue was selected for immunohistochemical visualization. Colorectal tissue from tumour-free regions was used to detect the immunophenotype of non-neoplastic control tissues.

For IHC, 3-µm-thick sections were cut on electrostatic glass slides (Histobond, Marienfeld, Germany). After deparaffinization and rehydration, antigen retrieval was performed in a microwave oven (3 × 5 min) using a basic TEG (pH 9.0) buffer, followed by blocking of endogenous peroxidase (Sigma-Aldrich). The sections were incubated with primary antibodies (full antibody characteristics and dilutions is described in Table 1.1) at room temperature. Bound antibodies were detected by the enzyme-conjugated polymeric visualization system EnVision, linked with horseradish peroxidase using 3,3′-diaminobenzidine as the chromogen. The stained slides were covered by cover glass (Biosigma). All IHC reagents were produced by DakoPositive and negative quality controls were invariably performed and reacted appropriately.

Table 1.1

Characteristics of primary antibodies

Antigen	Antibody characteristics	Clone	Dilution	Incubation min.	Expression pattern
E- cadherin	MMAH	NCH-38	1:50	60	Membranous
Vimentin	MMAH	Vim 3B4	1:200	60	Membranous
CD44	MMAH	DF1485	1:50	60	Membranous
β-catenin	MRAH	β-catenin-1	1:50	60	Membranous
N-cadherin	MMAH	6G11	1:50	60	Membranous
CD8	MRAH	C8/144B	1:50	60	Nuclear

Table 1.1 continued

Antigen	Antibody characteristics	Clone	Dilution	Incubation min.	Expression pattern
MMR proteins					
MSH2	MMAH	FE11	1:100	20	Nuclear
MSH6	MMAH	EP49	1:100	20	Nuclear
MLH1	MMAH	ES05	1:50	20	Nuclear
PMS2	MMAH	EP51	1:40	30	Nuclear

Abbreviations: MMAH, monoclonal mouse antibody against human antigen; MRAH, monoclonal rabbit antibody against human antigen

Expression of immunohistochemical markers was evaluated by light microscopy using high-power total magnification of 400× (i.e., 40× objective and 10× ocular lenses; 0.65 mm^2 per field). The expression of IHC markers – E-cadherin, vimentin, N-cadherin, β-catenin, and CD44 – and MMR proteins were assessed based on the intensity and the extent. The expression intensity was evaluated on a scale ranging from 0 to 3 as follows: 0, no expression; 1 weak expression; 2, moderate expression; and 3, strong expression (Figure 1.2) The relative extent (%) was measured as the fraction of cancer cells expressing the given marker at the given intensity. The final IHC score was calculated as the sum of the mathematical products of intensity and the relative extent. Subsequently, the MMR protein expression was reclassified as low versus high by using the median value as the cut-off threshold: 1.39 for MSH2, 1.9 for MSH6, 1.8 for PMS2, and 1.43 for MLH1. Additionally, the expression of MMR proteins was evaluated as a binary variable (complete loss vs. presence).

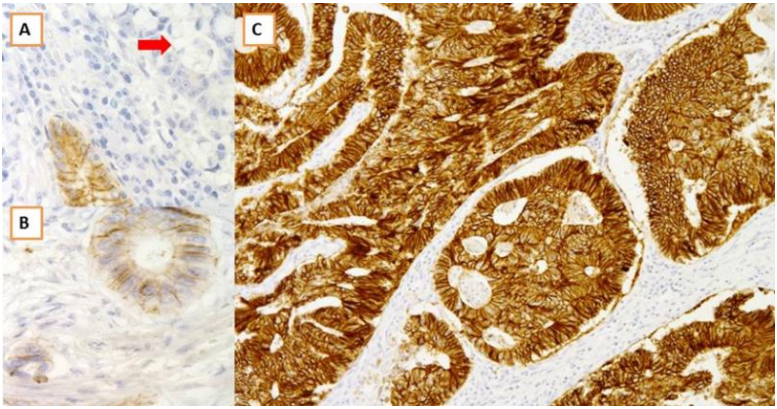

Figure 1.2 **Immunohistochemical marker expression evaluation**

A – total loss of E-cadherin expression in colorectal cancer (red arrow), original magnification (OM) 400×; B – weak E-cadherin expression in colorectal cancer cells, OM 400×; C – moderate and strong E-cadherin expression in colorectal cancer.

OM 100×. Images taken by Inese Briede.

For CD8+ tumour-infiltrating lymphocytes, the cell number per mm^2 was counted via Diapath in the tumour centre and the invasive margin, as described in previous studies (Lea et al., 2021; Ueno et al., 2014).

1.6 Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics Version 22.0 (IBM Corp., Armonk, NY, USA). The normality of the data was checked with the Shapiro-Wilk test. The descriptive data are expressed as the mean ± standard deviation (SD), the median with the interquartile range (IQR), or the relative frequency with the 95 % confidence interval (CI). Categorical data are presented as frequencies. For mean values and frequencies, 95 % CI were calculated. Because the data were not normally distributed, non-parametric methods, including the Mann-Whitney test, Spearman's rank correlation, and Pearson's chi-square were used for analytical statistics.

The Kruskal-Wallis one-way analysis of variance by ranks followed by the post hoc analysis with Bonferroni correction was applied to determine differences between three or more groups. A two-tailed $p < 0.05$ was considered statistically significant.

2 Results

2.1 Patients and surgical approach

2.1.1 Study group characteristics

The study included 553 consecutive primary CRC cases collected from pathology examination reports from 2011 to 2014. There were 258 male patients (46.6–95 % CI [42.5–50.8]) and 295 female patients (53.4 % [49.2–57.5]). The patient age ranged from 33 to 97 years. For the entire study group, the mean ± SD age was 68.8 ± 10.8 years (95 % CI [67.9–69.7]), with a median age of 71 years (IQR 15). Most of the patients were elderly: 93.0 % [90.5–94.8] were older than 50 years. Only 39/553 cases were patients younger than 50 years, including 18/39 men (46.2 % [31.6–61.4]) and 21/39 women (53.8 % [38.6–68.4]).

2.1.2 Gross findings

The majority of the tumours (278/392, 70.9 % [67.0–74.5]) were located in the left part of large bowel. In patients younger than 50 years, left-sided tumours were present in 31/39 cases (79.5 % [64.5–89.2]).

In 23/553 cases (4.2 % [2.8–6.2]), simultaneous CRC was found nearby, and it was found significantly ($p < 0.01$) more often with left-sided tumours (18/23 cases, 78.3 % [58.1–90.3]). The presence of simultaneous CRC differed significantly based on age ($p = 0.04$): In patients younger than 50 years, simultaneous CRC was found 4/39 cases (10.3 % [4.1–23.6]), while in patients older than 50 years it was present in 19/495 cases (3.7 % [2.4–5.7]).

In 155/553 cases (28.0 % [24.4–31.9]), synchronous adenoma was found within the operation material. Histologically, most of synchronous adenomas comprised tubular morphology, appearing in 82/155 cases (52.9 % [45.1–60.6]),

while villous adenomas were found in 26/155 cases (16.8 % [11.7–23.4]) and tubulovillous adenoma was found in 46/155 cases (29.7 % [23.1–37.3]).

The tumour volume was calculated by using the given 3D tumour measurements. Overall, precise measurements for tumours were present in 525/553 cases (94.9 % [92.8–96.5]), with an average size of 23.8 [19.7–27.8] cm^3. There was statistically significant difference ($p < 0.01$) in tumour size between right side tumours, where mean size was 29.4 [23.0–35.9] cm^3, and left side tumours where mean tumour size was 21.4 [16.3–26.5] cm^3. There was no statistically significant difference between tumours according to patients gender ($p=0.41$) or age ($p=0.75$).

Gross description of CRC revealed presence of circular tumour within 256/524 or 48.8 % [44.6–53.1] of cases, and showed statistically significant difference ($p < 0.05$) in frequency between the right (64 (25.0 % [20.1–30.7]) and the left (192 (75.0 % [69.3–79.9]) operation side.

Perforation was present in 38/553 cases (6. 9 %, [5.0–9.3]) cases, and it was significantly more common in left-sided tumours ($p < 0.01$), where it was present in 29/38 cases (76.3 % [60.8–87.0]).

2.2 Morphology

2.2.1 Characteristics of primary CRC

Regarding the morphology, colorectal adenocarcinoma (Figure 2.1 A) was found in 491/553 cases (88.8 % [85.9–91.2]), mucinous adenocarcinoma (Figure 2.1 B) in 53/553 cases (9.6 % [7.4–12.3]), and primary colorectal signet ring cell carcinoma (Figure 2.1 C) in only 7/553 cases (1.2 % [0.6–2.6]).

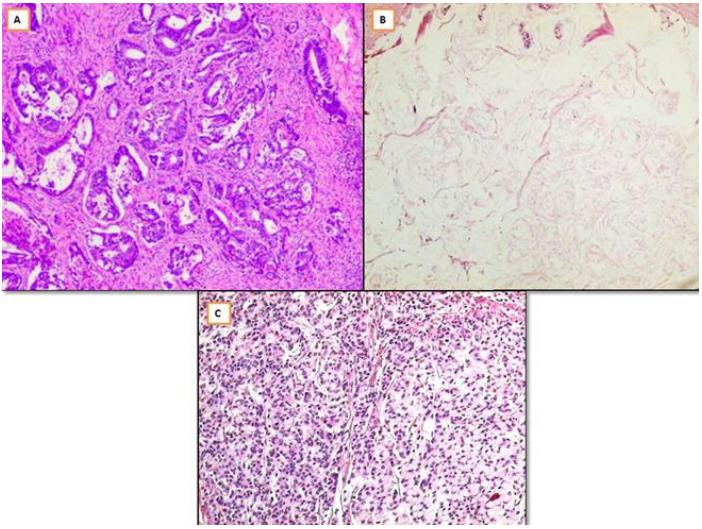

Figure 2.1 **Morphology of CRC in a study group**

A – moderately differentiated colorectal adenocarcinoma, HE stain, OM 40×;
B – mucinous carcinoma; C – signet ring cell carcinoma.

The tumour volume was significantly different depending on the CRC histology ($p < 0.01$). The mean tumour volume was 21.0 [17.4–24.6] cm^3 for adenocarcinoma, 45.3 [20.9–69.6] cm^3 for mucinous carcinoma, and 26.8 [4.2–49.4] cm^3 for signet ring cell carcinoma.

Evaluating the T parameter, locally advanced tumours predominated: pT3 carcinoma represented 274/553 cases (49.6 % [45.4–53.7]), pT4 represented 197/553 cases (35.6 % [31.7–39.7]). By grade, moderately differentiated (G2) cancers constituted 354/553 cases (64.0 % [59.9–67.9]), and high-grade (G3) carcinoma was found in 142/553 cases (25.7 % [22.2–29.5]).

Regarding lymph node involvement, metastases in lymph nodes (pN+) were found in 224/533 cases (40.5 % [36.5–44.6]). In 56/533 cases (10.1 % [7.9–12.9]) only tumour deposits (pN1c) were found within pericolonic or perirectal adipose tissue. The median number of retrieved lymph nodes was 11 (IQR 8). pTN and the tumour grade are summarized in Table 2.1.

Table 2.1

Characteristics of pT, pG, and lymph node status in study group

Variable	Count	Proportion, %	95 % confidence interval
pT			
pT1	16	2.9	1.8–4.6
pT2	66	11.9	9.5–14.9
pT3	274	49.6	45.4–53.7
pT4	197	35.6	31.7–39.7
pN			
pN0	273	49.4	45.2–53.5
pN1	156	28.2	24.6–32.1
pN1c	56	10.1	7.9–12.9
pN2	68	12.3	9.8–15.3
Grade			
G1	56	10.1	7.9–12.9
G2	354	64.0	59.9–67.9
G3	142	25.7	22.2–29.5
G4	1	0.2	0.0–1.0

Tumours with low differentiation (pG3) showed a significant association ($p < 0.01$) with the tumour volume, compared with moderate and well differentiated tumours. Mean tumour volume was 36.8 [25.0–48.6] cm^3 in pG3, 14.6 [7.4–21.8] cm^3 in pG1 and 20.1 [16.1–24.2] cm^3 in pG2 tumours.

There was also a significant difference according to tumour size and pT for left-sided tumours: pT3 and pT4 tumours had a greater volume ($p < 0.01$). The results are summarized in Table 2.2.

Table 2.2

CRC volume differences according tumour side and pT

pT	Mean tumour volume, cm^3; 95 % CI			
	Right side	P value	Left side	P value
pT1	39.82 [0.0–120.47]	0.051	11.11 [0.0–23.24]	**0.006**
pT2	11.93 [4.03–19.8]		8.76 [6.12–11.39]	
pT3	30.56 [20.68–40.44]		23.35 [15.27–31.43]	
pT4	29.41 [21.74–37.07]		24.42 [15.32–33.52]	

Colorectal adenocarcinoma was mostly detected at pT3 (249/491 cases, 50.7 % [46.3–55.1]) and pT4 (166/491 cases, 33.8 % [29.7–38.1]). Signet ring cell carcinoma was mostly detected at pT4 (6/7 cases, 85.7 % [48.7–97.4]), indicating its advanced growth.

In the whole study group, tumour invasion in lymphatic vessels was found in 352/553 cases (63.6 % [59.6–67.5]). Perineural growth occurred in 277/553 cases (50.1 % [45.9–54.2]), while intraneural growth occurred in 172/553 cases (31.1 % [27.4–35.1]). Overall, there was no significant difference in local structure involvement between right- and left-sided tumours ($p > 0.05$).

Overall, tumour necrosis was present in 295 cases, with most of the carcinomas comprising a moderate amount of necrosis, which was present in 146/295 cases (49.5 % [43.8–55.2]), while extensive amount of necrosis was observed only in 45/295 cases (15.3 % [11.6–19.8]). The mean extent of necrosis was significantly different in terms of pT ($p = 0.04$), pN ($p = 0.02$), and pG ($p < 0.01$), as it was more extensive in tumours with a higher stage and lower differentiation. The full results are described in Table 2.3. Perforation within the tumour showed no association with extent or presence of necrosis ($p = 0.33$). There were also no significant differences in the extent of necrosis and histological type of CRC.

Table 2.3

Extent of necrosis (mean %) in CRC according to pT, pN and tumour grade

Parameter	%	95 % CI	p
pT			
pT1	12.5	0.0–107.8	**0.04**
pT2	9.6	6.1–13.1	
pT3	14.5	12.5–16.4	
pT4	16.6	13.9–19.4	

Table 2.3 continued

Parameter	%	95 % CI	p
pN			
pN0	12.8	10.7–14.8	**0.02**
pN+	16.7	14.2–19.2	
pG			
pG1	9.3	5.2–13.3	**0.004**
pG2	13.4	11.8–15.0	
pG3	19.9	16.1–23.7	

2.2.2 Characteristics of peritumourous inflammation

Analysis of an inflammatory reaction within peritumoural tissue showed weak inflammation in 240/553 cases and complete absence of inflammation in 52/553 cases. After re-distribution based on the Klintrup-Mäkinen score, 292/533 cases (52.8 % [48.6–56.9]) had low-grade inflammation. High-grade inflammation (including 204 cases of moderate inflammation and 57 cases of severe inflammation) was observed in total in 261/533 cases (47.2 % [43.1–51.4]). These groups showed no statistically significant age differences ($p = 0.13$).

Regarding tumour volume, there was no significant difference ($p = 0.46$) between tumours with high-grade inflammation (23.6 [18.3–28.8] cm^3) and low-grade inflammation (23.9 [17.9–30.0] cm^3). There were significant differences (Table 2.4) regarding pT distribution ($p = 0.002$) and status of regional lymph nodes, reflected by pN ($p < 0.001$) in relation to low- and high-grade inflammation. However, there was no significant difference between the degree of inflammation and pG ($p=0.07$).

Table 2.4

Characteristics of pT, pN and pG in a study group

Parameter	Low-grade inflammation		High-grade inflammation		p
	Count	F, % [95 % CI]	Count	F, % [95 % CI]	
pT					
pT1	6	2.1 [0.9–4.4]	10	3.8 [2.1–6.9]	**0.002**
pT2	29	9.9 [7.0–13.9]	37	14.2 [10.5–18.9]	
pT3	132	45.2 [39.6–50.9]	142	54.4 [48.3–60.3]	
pT4	125	42.8 [37.3–48.5]	72	27.6 [22.5–33.3]	
pN					
pN0	118	40.4 [34.9–46.1]	155	59.4 [53.3–65.2]	< **0.001**
pN+	174	59.6 [53.9–65.1]	106	40.6 [34.8–46.7]	
Grade of the carcinoma (G)					
G1	26	8.9 [6.1–12.8]	30	10.3 [7.3–14.3]	0.07
G2	178	61.0 [55.3–66.4]	176	60.3 [54.6–65.7]	
G3	87	29.8 [24.8–35.3]	55	18.8 [14.8–23.7]	
G4	1	0.3 [0.0–2.1]	0	0.0 [0.0–1.6]	

Assessing the morphological manifestations of the invasive growth, tumours surrounded by low-grade peritumoural inflammation significantly more frequently featured invasion in lymphatic vessels (p = 0.003) (69.9 % [64.4–74.8] vs 56.7 [50.6–62.6] in high-grade inflammation), as well as intraarterial (p = 0.012) (5.1 % [3.1–8.3] vs 1.5 % [0.6–3.9]), intravenous (p = 0.017) (26.4 % [21.6–31.7] vs 19.2 % [1.8–24.4]), perineural (p = 0.001) (57.2 % [51.5–62.7] vs 42.1 % [36.3–48.2]), and intraneural (p = 0.01) (35.3 % [30.0–40.9] vs 26.4 % [21.5–32.1]) growth (Figure 2.2 and 2.3).

The degree of inflammation was not statistically significantly different between right- or left-sided colorectal carcinomas (p = 0.18) or by the presence (p = 0.63) or extent (p = 0.11) of tumour necrosis.

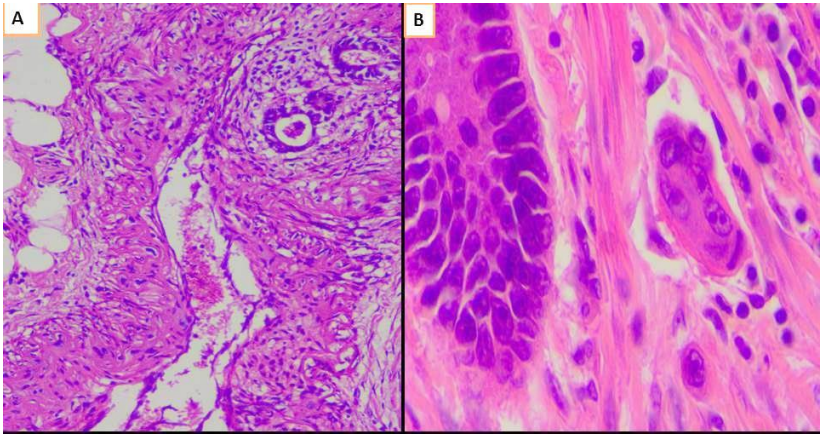

Figure 2.2 **Colorectal carcinoma invasion into local structures in tumours with low-grade inflammation**

A – invasion into an artery wall, with no colorectal carcinoma in its lumen, haematoxylin and eosin (HE) staining, original magnification (OM) 100×;
B – colorectal carcinoma complex in a lymphatic vessel, HE staining, OM 400×.

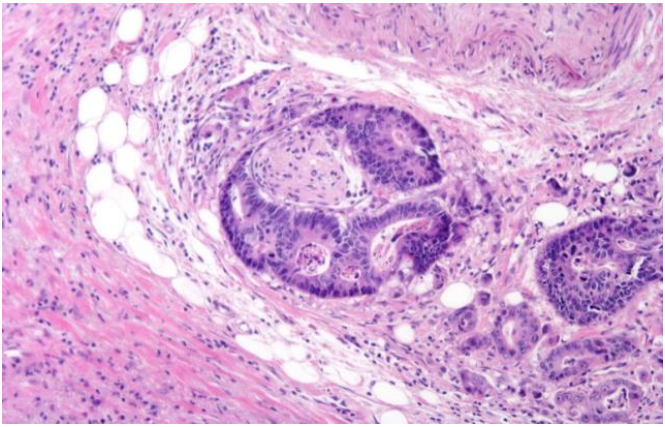

Figure 2.3 **Perineural invasion in presence of low-grade inflammation in colorectal carcinoma, haematoxylin and eosin staining**

Original magnification 40×.

CLR was found in 193/553 cases (34.9 % [31.0–39.0]) cases. Most of these tumours (165/193, 85.5 % [79.8–89.7]) showed a low density of lymphoid follicles.

There were no significant differences in the distribution of CLR density by cancer location (right versus left side of the large bowel), pT, pN, grade, tumour invasion into surrounding structures (blood or lymphatic vessels, nerves), or necrosis.

In contrast, there were significant differences between the presence and absence of CLR regarding the manifestations of invasive growth (Table 2.5).

Table 2.5

Association between presence of CLR and manifestations of invasive growth of colorectal carcinoma

Type of invasion	Number of cases; frequency, % [95 % CI]		p
	CLR present	CLR absent	
Lymphatic invasion	110 56.9 [49.9–63.8]	242 67.2 [62.2–71.9]	< **0.001**
Perineural invasion	85 44.0 [37.2–51.1]	192 53.3 [48.2–58.4]	< **0.001**
Intraneural invasion	47 27.3 [21.2–34.3]	125 72.7 [65.6–78.8]	< **0.001**
Intravenous invasion	31 24.4 [17.8–32.6]	96 75.6 [67.4–82.2]	< **0.001**

Inflammatory cell subpopulation analysis highlighted several significant associations between the density of certain tumour-infiltrating cells and the invasive capacity of the carcinoma, reflected by the manifestation of invasive growth (present vs. absent). There were significant differences regarding the number of neutrophils in cases with perineural ($p = 0.04$) and lymphatic ($p = 0.01$) invasion (Table 2.6).

Table 2.6

Inflammatory cell subpopulation analysis: neutrophil leucocytes

Type of identified invasion	Density of inflammatory cells: number of cases; proportion, % [95 % CI]				
	Absent	**Mild**	**Moderate**	**High**	**p value**
Neutrophilic leukocytes					
Perineural	202; 72.9 [67.4–77.8]	52; 18.8 [14.6–23.8]	17; 6.1 [3.8–9.7]	6; 2.2 [0.9–4.8]	**0.04**
Intraneural	126; 73.3 [66.2–79.3]	32; 18.6 [13.5–25.1]	12; 7.0 [3.9–11.9]	2; 1.2 [0.1–4.4]	0.13
Lymphatic	265; 75.3 [70.5–79.5]	57; 16.2 [12.7–20.4]	23; 6.5 [4.4–9.7]	7; 2.0 [0.9–4.1]	**0.01**
Intravenous	103; 81.1 [73.4–87.0]	16; 12.6 [7.8–19.6]	4; 3.2 [1.0–8.1]	4; 3.2 [1.0–8.1]	0.27
Intraarterial	16; 84.2 [61.6–95.3]	2; 10.5 [1.7–32.6]	0; 0.0 [0.0–19.8]	1; 5.3 [0.0–26.5]	0.48

Lymphocyte density differed in tumours with perineural ($p < 0.01$) and lymphatic ($p = 0.03$) invasion, but showed no significant difference in terms of vascular invasion and intraneural tumour growth. Table 2.7 shows a summary of lymphocyte density in a presence of invasions.

Table 2.7

Inflammatory cell subpopulation analysis: lymphocytes

Type of identified invasion	Density of inflammatory cells: number of cases; proportion, % [95 % CI]				
	Absent	**Mild**	**Moderate**	**High**	**p value**
Lymphocytes					
Perineural	32; 11.6 [8.3–15.9]	147; 53.1 [47.2–58.9]	87; 31.4 [26.2–37.1]	11; 4.0 [2.2–7.1]	**0.003**
Intraneural	20; 11.6 [7.6–17.4]	92; 53.5 [46.0–60.8]	53; 30.8 [24.4–38.1]	7; 4.1 [1.8–8.3]	0.052

Table 2.7 continued

Type of identified invasion	Density of inflammatory cells: number of cases; proportion, % [95 % CI]				
	Absent	**Mild**	**Moderate**	**High**	**p value**
Lymphatic	39; 11.1 [8.2–14.8]	184; 52.3 [47.1–57.4]	115; 32.7 [28.0–37.7]	14; 4.0 [2.3–6.6]	**0.03**
Intravenous	14; 11.0 [6.6–17.8]	66; 52.0 [43.4–60.5]	43; 33.9 [26.2–42.5]	4; 3.2 [1.0–8.1]	0.88
Intraarterial	3; 15.8 [4.7–38.4]	13; 68.4 [45.8–84.8]	3; 15.8 [4.7–38.4]	0; 0.0 [0.0–19.8]	0.09

Eosinophil absence was significantly associated with perineural invasion ($p = 0.01$) and invasion into lymphatic vessels ($p < 0.01$). Table 2.8 provides a full summary of the eosinophil density relationship to local structure invasion.

Table 2.8

Inflammatory cell subpopulation analysis: eosinophilic leucocytes

Type of identified invasion	Density of inflammatory cells: number of cases; proportion, % [95 % CI]				
	Absent	**Mild**	**Moderate**	**High**	**p value**
Eosinophilic leukocytes					
Perineural	207; 74.7 [69.3–79.5]	64; 23.1 [18.5–28.4]	6; 2.2 [0.9–4.8]	0; 0.0 [0.0–1.7]	**0.01**
Intraneural	128; 74.4 [67.4–80.4]	39; 22.7 [17.0–29.5]	5; 2.9 [1.1–6.8]	0; 0.0 [0.0–2.6]	0.31
Lymphatic	264; 75.0 [70.2–79.3]	82; 23.3 [19.2–28.0]	6; 1.7 [0.7–3.8]	0; 0.0 [0.0–1.3]	**0.008**
Intravenous	92; 72.4 [64.1–79.5]	32; 25.2 [18.4–33.4]	3; 2.4 [0.5–7.0]	0; 0.0 [0.0–3.5]	0.21
Intraarterial	17; 89.5 [67.4–98.3]	2; 10.5 [1.7–32.6]	0; 0.0 [0.0–19.8]	0; 0.0 [0.0–19.8]	0.36

2.3 Characteristics of immunohistochemistry markers in CRC

Immunohistochemistry was performed to 124 consecutive CRC cases, to illustrate the presence of CD8+ cytotoxic cells, epithelial mesenchymal transition and MMR proteins within study group.

2.3.1 Cytotoxic T cells (CD8)

There were significantly more CD8+ T cells ($p < 0.01$) in the invasive margin or peritumoural area compared with the tumour centre or intratumoural region. Within the peritumoural area, there was an average of 148 CD8+ T cells (range 4–417) (Figure 2.4), but within the intratumoural region, there was an average of 18 CD8+ T cells (range 0–120).

Regarding tumour histology, there were no significant differences in the number of CD8+ T cells amount in the peritumoural ($p = 0.23$) or intratumoural ($p = 0.41$) areas.

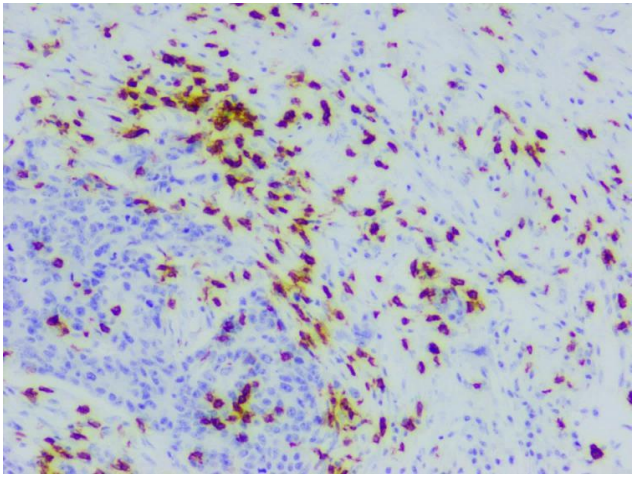

Figure 2.4 **CD8+ T cells in peritumourous area, immunoperoxidase** OM 100×.

There was a significant correlation between the peritumoural CD8+ T cell count and pT (r = -0.2, p = 0.03), but no significant correlation between the number of intratumoural CD8+ T cells and pT (p = 0.13). There was a significant difference between the number of peritumoural CD8+ T cells in tumours without lymph node metastasis (pN0) and in tumours with metastasis in lymph nodes (pN+) or separate tumour nodes in fat tissue (p = 0.05). There was no significant difference between the number of peritumoural and intratumoural CD8+ T cells according to the tumour grade (p = 0.93 and p = 0.10, respectively). Table 2.9 presents the CD8+ T cell relations to pT, pN, and tumour grade.

Table 2.9

Peritumourous and intratumourous CD8+ cytotoxic cell amount in relation to pT, pN and tumour grade

Parameter	PT CD8+ [95 % CI]	p value	IT CD8+ [95 % CI]	p value
pT				
pT1	198.5 [82.6–314.4]	**0.03**	21.5 [0.0–129.5]	0.13
pT2	153 [111.6–193.6]		26.0 [9.7–42.2]	
pT3	163 [141.5–185.0]		18.4 [12.7–24.1]	
pT4	121 [95.7–146.9]		12.9 [7.0–18.9]	
pN				
pN0	163.2 [140.2–186.2]	**0.05**	20.5 [13.9–27.1]	0.16
pN+	136.6 [113.4–160.0]		15.5 [9.5–21.6]	
pN1c	120.0 [65.8–174.3]		12.4 [5.1–19.7]	
pG				
pG1	100.0 [14.4–186.2]	0.93	12.0 [0.0–50.8]	0.10
pG2	151.6 [132.0–171.2]		15.6 [11.2–19.9]	
pG3	142.3 [114.9–169.8]		22.8 [13.2–32.4]	

Regarding the cytotoxic T cell distribution, there was a significant difference in the number of peritumoural CD8 cells in CRC with and without perineural (p = 0.02) (125.2 [103.1–147.3] and 160.2 [139.6–180.8], respectively) and intraneural (p = 0.02) (108.6 [69.8–147.3] and

153.8 [137.0–170.6], respectively) invasions, but there was no significant correlation with other local structure involvement.

2.3.2 Epithelial mesenchymal transition characteristics in the study group

E-cadherin, N-cadherin, β-catenin, vimentin, and CD44 were detected with IHC to describe EMT (Figure 2.5.). The overall CD44 score was 1.34 (95 % CI 1.21–1.47), reaching 1.28 (95 % CI 1.15–1.41) in adenocarcinomas and 2.00 (95 % CI 1.61–2.38) in mucinous carcinomas ($p = 0.02$). The overall E-cadherin score was 1.86 (95 % CI 1.78–1.94): 1.91 (95 % CI 1.83–1.98) in adenocarcinomas and 1.48 (95 % CI 1.23–1.73) in mucinous carcinomas ($p = 0.001$). The overall β-catenin score was 2.59 (95 % CI 2.50–2.67): 2.60 (95 % CI 2.50–2.69) in adenocarcinomas and 2.69 (95 % CI 2.47–2.92) in mucinous carcinomas ($p = 0.41$). The N-cadherin score was 1.74 (95 % CI 1.53–1.92), reaching 1.78 (95 % CI 1.58–1.98) in adenocarcinomas and 1.49 (95 % CI 0.55–2.43) in mucinous carcinomas ($p = 0.37$).

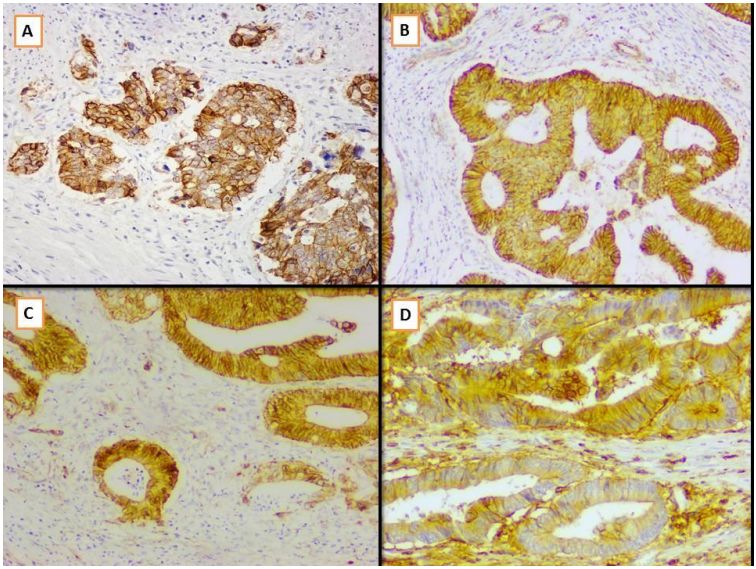

Figure 2.5 **Epithelial-mesenchymal transition marker immunohistochemistry in colorectal carcinoma**

A – E-cadherin expression, immunoperoxidase staining, original magnification (OM) 40×; B – N-cadherin expression, immunoperoxidase staining, OM 40×; C – heterogenous β-catenin expression, immunoperoxidase staining, OM 100×; D – heterogenous CD44 expression, immunoperoxidase staining, OM 100×.

Immunohistochemically, CD44 expression showed significant differences regarding tumour side ($p < 0.01$) (1.21 [1.07–1.36] in left vs 1.62 [1.38–1.87] on right side tumours). Moreover, CD44 expression was significantly higher in pN0 tumours than in pN+ tumours ($p = 0.02$) (1.46 [1.28–1.64]) vs. 1.18 [0.96–1.39]). In contrast, CD44 levels did not differ based on the presence of sCRC ($p=0.40$), pT ($p=0.23$), grade ($p=0.07$), or manifestations of invasive growth ($p>0.05$). There were also no differences in CD44 expression between the inflammation grades. Table 2.10 presents the CD44 expression characteristics according the degree of inflammation.

Table 2.10

CD44 expression characteristics according inflammation

Parameter	CD44		
	Score	95 % CI	p
Inflammation			
Low-grade	1.23	1.06–1.40	0.23
High-grade	1.42	1.23–1.61	
Neutrophilic leukocytes			
Low density	1.30	1.17–1.44	0.14
High density	1.55	1.23–1.88	
Eosinophilic leukocytes			
Low density	1.31	1.18–1.44	0.33
High density	1.58	1.05–2.10	
Lymphocytes			
Low density	1.26	1.09–1.42	0.33
High density	1.40	1.21–1.60	
Macrophages			
Low density	1.31	1.14–1.47	0.64
High density	1.38	1.19–1.57	
Crohn's like lymphoid reaction (CLR) by density of lymphoid follicles per mm			
Low density	1.46	1.25–1.67	0.93
High density	1.42	0.55–2.29	

β-catenin expression was significantly different in relation to pT, showing higher expression in more advanced tumours ($p = 0.007$): 2.92 [2.61–3.24] in pT1; 2.64 [2.47–2.81] in pT2; 2.65 [2.50–2.80] in pT3; 2.47 [2.34–2.60] in pT4. However, there were no expression differences regarding the presence of sCRC ($p=0.58$), local structure invasion ($p>0.05$), or involvement of lymph nodes ($p=0.42$). Interestingly, β-catenin expression levels were affected by the number of neutrophils, with lower β-catenin expression in cases with a low neutrophil count ($p = 0.001$). Table 2.11 illustrates β-catenin expression according the degree of inflammation

Table 2.11

β-catenin expression characteristics according inflammation

Parameter	β-catenin		
	Score	**95 % CI**	**p**
Inflammation			
Low-grade	2.65	2.56–2.74	0.88
High-grade	2.54	2.39–2.69	
Neutrophilic leukocytes			
Low density	2.56	2.46–2.66	**0.001**
High density	2.88	2.81–2.94	
Eosinophilic leukocytes			
Low density	2.59	2.49–2.68	0.34
High density	2.71	2.42–2.99	
Lymphocytes			
Low density	2.64	2.55–2.73	0.68
High density	2.54	2.39–2.70	
Macrophages			
Low density	2.60	2.50–2.70	0.51
High density	2.59	2.40–2.77	
Crohn's like lymphoid reaction (CLR) by density of lymphoid follicles per mm			
Low density	2.57	2.42–2.72	0.06
High density	2.84	2.71–2.97	

N-cadherin expression differed significantly only in tumours with a synchronous lesion (p = 0.03) (0.80 [0.0–2.10]) vs tumours without sCRC (1.80 [1.61–1.99]), and based on pT (p = 0.03) (pT1: 1.10[0.00–15.1]; pT2: 1.87 [1.28–2.45]; pT3 1.97 [1.71–2.23]; pT4: 1.45[1.12–1.78]) and the eosinophil density (p = 0.02). Table 2.12 summarizes the N-cadherin expression levels according the degree of inflammation.

Table 2.12

N cadherin expression characteristics according inflammation

Parameter	N-cadherin		
	Score	**95 % CI**	**p**
Inflammation			
Low-grade	1.83	1.56–1.97	0.57
High-grade	1.69	1.40–1.98	

Table 2.12 continued

Parameter	N-cadherin		
	Score	95 % CI	p
Neutrophilic leukocytes			
Low density	1.77	1.56–1.97	0.72
High density	1.68	1.03–2.32	
Eosinophilic leucocytes			
Low density	1.84	1.65–2.03	**0.02**
High density	0.66	0.00–1.67	
Lymphocytes			
Low density	1.84	1.58–2.09	0.56
High density	1.67	1.37–1.97	
Macrophages			
Low density	1.82	1.60–2.05	0.51
High density	1.62	1.24–2.00	
Crohn's like lymphoid reaction (CLR) by density of lymphoid follicles per mm			
Low density	1.70	1.39–2.02	0.16
High density	2.40	2.08–2.71	

E-cadherin expression differed significantly based on grade (p = 0.001) and tumour side (p = 0.02) (1.91 [1.82–1.99] on left side CRC vs 1.72 [1.56–1.89] on right side CRC), but not by pT, pN, manifestations of invasive growth, or presence of sCRC. There was also a significant difference in E-cadherin expression and eosinophil density: E-cadherin was upregulated when there was a high density of eosinophils (p = 0.007). Table 2.13 provides a summary of E-cadherin expression according the degree of inflammation.

Table 2.13

E-cadherin expression characteristics according inflammation

Parameter	E-cadherin		
	Score	95 % CI	p
Inflammation			
Low-grade	1.87	1.76–1.97	0.90
High-grade	1.85	1.74–1.96	
Neutrophilic leucocytes			
Low density	1.86	1.77–1.94	0.95
High density	1.88	0.66–3.11	

Table 2.13 continued

Parameter	E-cadherin		
	Score	95 % CI	p
Eosinophilic leucocytes			
Low density	1.82	1.75–1.90	**0.007**
High density	2.31	1.88–2.10	
Lymphocytes			
Low density	1.85	1.75–1.96	0.85
High density	1.87	1.75–1.98	
Macrophages			
Low density	1.87	1.78–1.96	0.97
High density	1.84	1.70–1.98	
Crohn's like lymphoid reaction (CLR) by density of lymphoid follicles per mm			
Low density	1.84	1.75–1.94	0.38
High density	1.77	1.38–2.15	

Analysis of EMT marker expression cross-correlation showed significant differences in β-catenin and E-cadherin expression ($p = 0.018$) and between β-catenin and N-cadherin expression ($p = 0.000$). There were no other significant correlations.

In low-grade inflammation, there was a significant correlation between β-catenin and E-cadherin expression ($p < 0.05$) and between β-catenin and N-cadherin expression ($p < 0.01$). There were no other significant correlations for low grade inflammation. Interestingly, there were no significant correlations between EMT markers in high-grade inflammation.

2.4 Mismatch repair protein expression in the study group

MMR protein expression was detected in 118/124 cases (95.2 %, 95 % CI 89.8–97.8 %); 6/124 cases (4.8 %, 95 % CI 2.2–10.2 %) had total MMR protein loss. The mean ± SD expression was 1.43 ± 0.06 for MSH2, 1.79 ± 0.07 for MSH6, 1.46 ± 0.08 for PMS2, and 1.31 ± 0.07 for MLH1. The MMR protein expression was reclassified as low versus high by using the median value as the

cut-off threshold: 1.39 for MSH2, 1.9 for MSH6, 1.8 for PMS2, and 1.43 for MLH1.

There were significant differences in MSH6 ($p = 0.03$) and PMS2 ($p < 0.01$) expression based on the tumour histological type (Table 2.14). No statistically significant changes in MMR protein expression was found in an association to pT and pN parameters ($p>0.05$).

Table 2.14

MMR protein expression and CRC histogenesis

Histo-logical type	**MSH2 Score [95 % CI]**	**p value**	**MSH6 Score [95 % CI]**	**p value**	**PMS2 Score [95 % CI]**	**p value**	**MLH1 Score [95 % CI]**	**p value**
CRac	1.48 [1.35–1.60]	0.06	1.84 [1.69–1.98]	**0.03**	1.54 [1.39–1.70]	**0.002**	1.36 [1.21–1.50]	0.13
CRmac	1.14 [0.47–1.80]		1.65 [0.97–2.33]		0.72 [0.15–1.30]		0.87 [0.17–1.57]	
CRsrc	0.14 [0.0–1.53]		0.07 [0.0–0.39]		0.02 [0.0–0.21]		–	

MSH2 expression (Figure 2.6) was significantly different based on tumour grade ($p=0.02$) (pG1:1.30 [0.48–2.11]; pG2: 1.55 [1.41–1.70]; pG3: 1.17 [0.91–1.43]) and operated side ($p < 0.01$) (1.58 [1.44–1.72]) on the left side CRC vs 1.07 [0.81–1.32] on the right side CRC).

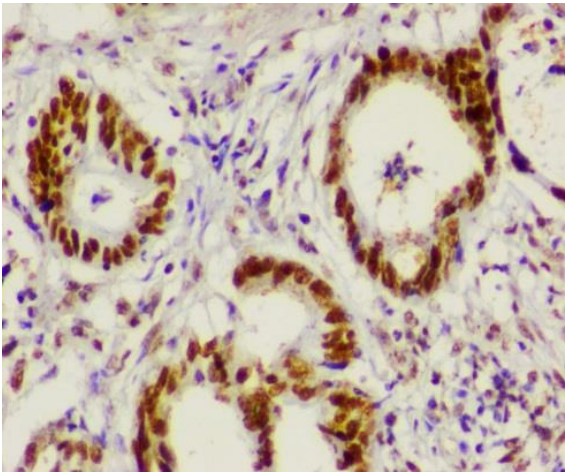

Figure 2.6 **MSH2 strong expression in CRC, immunoperoxidase** OM 200×.

MSH6 expression was significantly different ($p = 0.01$) according to tumour side: Left-sided tumours showed intense expression, while right-sided tumours showed slightly weaker expression. There was no significant difference in MSH6 expression in terms of pT, pN, and tumour grade.

Similarly to the MSH2 expression pattern, PMS2 expression was significantly different depending on tumour side ($p<0.001$) (1.70 [1.55–1.85] in the left CRC vs 0.84 [0.53–1.15] in the right side CRC) and pG ($p<0.001$) (pG1: 1.06 [0.0–2.60]; pG2: 1.69 [1.52–1.86]; pG3: 1.00 [0.70–1.29]).

MLH1 expression was significantly different ($p < 0.001$) depending on tumour side, with weak expression associated with right-sided tumours. MLH1 showed no expression changes according to tumour differentiation ($p=0.09$).

In cases with invasion into local structures, only MSH6 expression showed significant differences, specifically for intraneural invasion ($p = 0.05$). When stratifying the cases into high versus low MMR protein expression, there was only a significant difference for MLH1 in terms of lymphatic invasion ($r = 0.19$, $p = 0.04$)

After stratifying the cases into high versus low MMR protein expression, there was a significant difference in E-cadherin expression relative to MSH2 ($p<0.01$) (1.91 [1.81–2.01] vs 1.72 [1.61–1.82]) and PMS2 expression ($p=0.01$) (1.95 [1.85–2.05] vs 1.71 [1.60–1.82]; N-cadherin and β-catenin showed no significant differences in relation to MMR protein expression. CD44 only showed a significant difference ($p=0.05$) with PMS2 expression (1.17 [1.00–1.34] vs 1.43 [1.24–1.62]).

There was no significant association between the grade of peritumoural inflammation according to the Klintrup-Mäkinen score and MMR protein expression. Individual cell composition analysis of peritumoural inflammatory infiltrate showed no significant differences for any peritumoural inflammatory cell and MSH2, MSH6 and PMS2 expression. Increased neutrophil density was associated with increased MLH1 expression, ranging from 1.26 [1.11–1.41] in cases with low-grade infiltration to 1.75 [1.46–2.04] in carcinomas showing a moderate to high neutrophil count. There were no other significant differences in MLH1 expression in relation to other inflammatory cells or CLR.

The number of peritumoural and intratumoural CD8+ cytotoxic T cells was not significantly different between the low and high MMR protein expression groups or complete loss of MMR (Table 2.15).

Table 2.15.

MMR proteins and CD8+ T cells in CRC

Parameter	PT CD8+, mean count [95 % CI]	p value	IT CD8+, mean count [95 % CI]	p value
MSH2				
Low MMR	140.9 [116.9–165.0]	0.31	14.8 [10.3–19.3]	0.25
High MMR	153.6 [132.2–175.1]		19.2 [13.0–25.6]	
MSH6				
Low MMR	134.1 [111.5–156.6]	0.17	16.7 [11.1–22.3]	0.63
High MMR	159.0 [137.0–181.0]		18.7 [12.7–24.7]	

Table 2.15 continued

Parameter	PT CD8+, mean count [95 % CI]	p value	IT CD8+, mean count [95 % CI]	p value
PMS2				
Low MMR	145.9 [122.4–169.3]	0.68	17.6 [11.8–23.4]	0.94
High MMR	151.2 [129.6–172.8]		18.0 [12.0–24.0]	
MLH1				
Low MMR	151.5 [128.1–174.9]	0.95	15.9 [10.4–21.4]	0.19
High MMR	148.5 [127.0–170.0]		19.9 [13.6–26.1]	
Loss of MMR				
Present	104.8 [11.6–198.0]	0.27	23.0 [0.0–76.5]	0.23
Absent	149.5 [133.6–165.3]		17.3 [13.4–21.3]	

3 Discussion

CRC remains one of the malignancies with the highest incidence as well as mortality (Siegel, Miller, & Jemal, 2020). Although there are prognostic factors of this malignancy that we cannot change, like patient age and tumour location, several factors (EMT, stemness, immune response) can be affected and altered with treatment. Factors driving the metastatic potential of CRC are thought to be EMT and CSCs (Fabregat et al., 2016; Fedyanin, Popova, Polyanskaya, & Tjulandin, 2017), but their potential interaction with each other as well other tumour-driving factors like inflammation is unclear.

The goal of this study was to determine the molecular mechanisms involved in CRC development and their connection with histopathological findings, EMT, and the inflammatory reaction.

3.1 Clinical and morphological findings

Gender. In some Western countries, the CRC incidence does not differ between female and male patients (Abotchie, Vernon, & Du, 2012; Ghebrial et al., 2021). The current study showed slight predominance of female patients, who constituted 53.4 % [49.2–57.5] of the study group. The differences between these studies could be explained by the study design, as this study did not include patients who had not received surgical treatment because of advanced tumour spread or significant comorbidities (e.g., cardiovascular).

Age. CRC is known to appear mostly after the age of 50 years, although lately there has been an increased tendency to identify CRC in patients younger than 50 years and a decrease in the number of elderly patients, especially in Western countries (Vuik et al., 2019; Wong et al., 2021). In the current study, 93 % (95 % CI 90.5–94.8 %) of the study group was over 50 years old, with a median age of 71 (IQR 15) years. In Sweden, the median age for male patients

with CRC is 70 years, but for females it is 69 years (Ghazi et al., 2012). Although other studies indicate that most patients with CRC are elderly, the average age of diagnosis in other studies varies up to the age of 70 years (S. Li et al., 2019; Lim, Kuk, Kim, & Shin, 2017).

Operated side. In the current study, left-sided CRC was more common, occurring in 70.9 % of cases. This result is similar to a recent study done in Italy where right-sided tumours occurred in 30.1 % of cases in a study group comprising more than 29,000 patients with CRC (Mangone et al., 2021).

Simultaneous tumours. The current study indicated that patients with left-sided CRC more often tend to have sCRC, as their presence in the left part of the bowel was observed in 78.3 % of patients with sCRC, and overall sCRC occurred in 4.2 % of all cases. Lee et al., also found that sCRC was more frequent on the left side, although the overall incidence of sCRC was lower, only 2.6 % in their study group, and overall only 33.1 % of tumours were located on the right side (B. C. Lee et al., 2017).

In the current study, 155/553 cases (28 %) presented synchronous adenomas. This result is very similar to other countries – the range of synchronous adenomas in patients with CRC varies from 25 % to 39 % (S. Li et al., 2019; Sun et al., 2020). The morphology of synchronous adenomas in the current study was slightly different from other studies. Tubular adenoma was detected in 52.9 % of cases.

Tumour size. The significance of tumour size in CRC has been widely disputed. In a recent study, patients with stage T1/2 N0 rectal tumours with a larger volume had a lower disease-free survival rate than patients with smaller volume rectal tumours (80.4 % vs. 89.6 %, $p = 0.042$) (Jiang et al., 2018). There was a significant difference in the tumour volume depending on its location: left-sided tumours were smaller than right-sided tumours (21.4 [95 % CI 16.3–26.5] cm^3 vs. 29.4 [95 % CI 23.0–35.9] cm^3, respectively). In other studies, left-sided

tumours also tended to be smaller. In a large retrospective studies done in Korea, the mean ± SD side of a left-sided CRC was 4.3 ± 2.2 cm and 4.4 ± 2.3 cm, compared with 5.1 ± 2.7 cm and 5.6 ± 3.1 cm for right-sided CRC (Kwak et al., 2021; Lim et al., 2017).

Morphology. In current study, colorectal adenocarcinoma was found in 88.8 % of cases. This finding is consistent with other studies, namely that it is the most common morphological type of CRC. In other studies, colorectal adenocarcinomas has varied from 80 % to 90 % (Betge et al., 2012). The prevalence of mucinous carcinomas (9.6% of cases) is lower than in studies done in Romania (19.3 % of cases) (Mogoantă, Vasile et al., 2014) and Austria (11 % of cases) (Betge et al., 2012; Mogoantă et al., 2014).

This research also similarly to other studies (Z.-P. Li et al., 2020) indicates that mucinous carcinomas tend to have larger size then non-mucinous carcinomas, as in this current research mucinous CRC mean tumour volume comprised 45.3 [20.9–69.6] cm3. In compartment mean non-mucinous CRC volume in this study was 21.0 [17.4–24.6] cm3.

pT: The study cohort showed a remarkable predominance of locally advanced tumours: pT3 carcinoma represented 49.6 % (95 % CI 45.4–53.7 %) of cases and pT4 represented 35.6 % (95 % CI 31.7–39.7 %) of cases, but pT2 only represented 11.9 % (95 % CI 9.5–14.9 %) of cases. In other countries, researchers have reported fewer pT4 tumours; this difference could be explained by delayed diagnosis of CRC, given that in Latvia the response to screening is < 12 % (Senore et al., 2019). Betge et al. reported that pT4 comprised only 17.0 % of cases; the majority of the cases they included in their study were pT3 (57.2 %), and the percentages of pT1 and pT2 cases were higher than in the current study (Betge et al., 2012).

Regarding histology, in the current study most colorectal adenocarcinoma cases (50.7 %) were detected at pT3 and pT4 (33.8 %), while only 3.3 % and 12.2 % of cases were pT1 and pT2, respectively. Mucinous carcinomas were found equally at the pT3 and pT4 stages (43.4 % and 45.3 %, respectively). However, signet ring cell carcinoma was mostly detected at pT4 (85.7 %), indicating its aggressive growth.

Previous studies have shown very similar percentages of mucinous carcinoma cases in the colon. Park et al. reported that 90.9 % of mucinous carcinoma was pT3 and pT4 (J. S. Park et al., 2015). Moreover, they observed clinical signs of bowel obstruction in only 13.9 % of cases and bowel perforation in 1.5 % of cases. These findings indicate the problem of mucinous tumour detection, as there are no symptoms in most of the cases.

There was a higher percentage of advanced adenocarcinoma in the current study compared with previous studies. More than half of the patients (50.7 %) had pT3 tumours and 33.8 % had pT4 tumours. In another study, pT4 colorectal adenocarcinoma comprised only 8 % of cases, while pT3 was found in 71 % of cases (Hosseini et al., 2017).

pN: In the current study, 40.5 % of cases were pN+ and 49.4 % of cases were pN0. These percentages are different from patients in other studies, where pN0 was slightly more common (54.0–55.9 %) (Betge et al., 2012; Ptok, Meyer, Croner, Gastinger, & Garlipp, 2022). Although the number of pN0 patients is similar, the current study had a higher number of patients with advanced-stage disease.

Another indicator of more advanced tumours in the study group is the presence of tumour nodules in fat tissue (pN1c), identified in 10.1 % of cases. Research has shown that patients with pN1c CRC should be considered high risk and treated with adjuvant chemotherapy, as their presence is associated with

higher pTNM stage, vascular emboli, and lower 3-year overall survival compared with pN1a CRC (81.8 % vs. 89.1 %) (Bouquot et al., 2018).

pG: There were a relatively high number of tumours with low differentiation compared with other studies. Specifically, 64.0 % of cases were pG2 and 25.7 % were pG3. However, Vayrynen et al. found that only 12.9 % of their cases were pG3 tumours (Vayrynen et al., 2016). Lee et al. also identified fewer pG3 tumours: 80.6 % 5.4 % of single CRC cases were pG2 and pG3, respectively, and 85.7 % and 4.6% of sCRC cases were pG2 and pG3, respectively (B. C. Lee et al., 2017).

Invasions. Local structure invasions are one of the main prognostic factors in CRC. These factors have been widely studied, and their presence is associated with low survival rates in CRC (Hogan et al., 2015; Kuan et al., 2019).

In the current study lymphovascular invasion was detected in 63.6 % of cases, perineural invasion in 50.1% of cases, and intraneural growth in 31.1 % of cases. In other studies, lymphovascular invasion varies from 19.7 % to 27.8 % (B. C. Lee et al., 2017; J. S. Park et al., 2015), and perineural invasion varies from 5.8 % to 14.7 % (B. C. Lee et al., 2017; J. S. Park et al., 2015). The incidence of reported venous invasion varies from 11 % to 89.5 % (Kojima et al., 2013). These findings indicate high numbers of advanced-stage tumours in this study and could be explained by a late diagnosis of CRC.

Tumour necrosis affects patient survival and tumour progression in CRC (Vayrynen et al., 2016) as well as other cancer types, including nasopharyngeal carcinoma (Liang et al., 2021) and lung carcinoma (Oiwa et al., 2021). In the current study, necrosis was present in 295 cases, presenting mostly a moderate amount of necrosis (49.5 %, 95 % CI 43.8–55.2 %). Its presence was significantly higher in pT4 tumours, where its mean extent was 16.6% of the tumour mass ($p = 0.04$), in pN+ tumours ($p = 0.02$) and in low-differentiated tumours ($p = 0.004$). These results are somewhat similar to

other studies. Vayrynen et al. found that the necrotic area was associated with a higher pT stage and showed variations regarding lymph node involvement: There was a median of 25.0 % necrosis in pN2 tumours compared with 7.0 % in pN0 tumours (Vayrynen et al., 2016).

3.2 Inflammation in CRC

The current study showed a slight predominance (52.8 %) of CRC cases with low-grade peritumoural inflammation. There was no significant difference ($p = 0.13$) between the mean ages of patients with low-grade (68.1, 95 % CI 66.9–69.3) and high-grade (69.6, 95 % CI 68.2–70.9) peritumoural inflammation. There were no significant differences regarding tumour volume and grade of local inflammation, indicating that the local immune response could potentially play a different role in CRC. Previous research has shown ambiguous results, as mostly systemic inflammation effects have been evaluated, namely C-reactive protein, fibrinogen, and other acute phase proteins (Koper-Lenkiewicz, Dymicka-Piekarska, Milewska, Zińczuk, & Kamińska, 2021; Rasic, Radovic, & Aksamija, 2016), but local immune response are overlooked in just few studies.

Peritumoural inflammation was significantly associated with the depth of local invasion as well as the regional lymph node status. Low-grade inflammation was frequently observed in pT4 cases (63.5 %, 95 % CI 56.5–69.9 %) and was significantly less common in pT3 cases (48.2 %, 95 % CI 42.3–54.1 %). An association between high-grade inflammation and T1–2 (opposed to T3-4) has been reported (Richards et al., 2012). Thus, the Klintrup-Mäkinen score has distinct associations with the full scope of pT, from the earliest pT parameters to advanced cases, which dominated in this study. Similarly, only 40.4 % (95 % CI 34.9–46.1 %) of patients with low-grade inflammation had pN0 CRC, in contrast to 59.4 % (95 % CI53.3–65.2 %) in those presenting with high-grade peritumoural inflammation.

These findings are consistent with Richards et al., who also reported a significant difference ($p = 0.0039$) between inflammation grade in pN0 versus pN+ (Richards et al., 2012). Given that pT and pN are the strongest prognostic factors, the significant association between high-intensity inflammation and less extensive cancer spread indirectly confirms the previously described association between high-grade inflammation based on the Klintrup-Mäkinen score and beneficial cancer-specific or overall survival.

There were significant differences between local structure involvement and the degree of peritumoural inflammation, as low-grade peritumoural inflammation more frequently featured invasion into lymphatic vessels ($p = 0.003$), arteries ($p = 0.012$), and veins ($p = 0.017$), as well as perineural ($p = 0.001$) and intraneural ($p = 0.01$) growth. These results are somewhat different from previous reports. In a study were the authors evaluated the MMR status in primary operable CRC in relation to local and systemic inflammation, there were no significant differences in local structure involvement when there was a high density of intratumoural lymphocytes (J. H. Park et al., 2016). However, in a recent study tumour-infiltrating lymphocytes were associated with the absence of venous, lymphatic, and perineural invasion (Jakubowska, Koda, Grudzińska, Lomperta, & Famulski, 2021). The differences in the results indicate complex mechanisms involved in the immune response, and this topic should be evaluated in greater depth.

Analysis of inflammatory cell subpopulations showed, that the density of eosinophils ($p = 0.008$), neutrophils ($p = 0.01$) and lymphocytes ($p = 0.03$) are associated with cancer invasion into lymphatic vessels. Perineural growth was associated with the same cellular players: lymphocytes ($p = 0.003$), eosinophils ($p = 0.01$) and neutrophils ($p = 0.04$). Previously, high numbers of stromal eosinophils in colorectal cancer have been reported to show association with lower tumour stage, better overall and cancer specific 5-year survival, reflected

by hazard ratios for death of 0.61 (95 % CI: 0.36–1.02; p=0.02) and 0.48 (95 % CI: 0.24–0.93; p=0.01), respectively (A. Prizment et al., 2016). Further, higher density of peritumoural eosinophils was significantly associated with lower pT, pN, and pG; absence of vascular invasion; and longer progression-free and cancer-specific survival (Harbaum et al., 2015).

The previous findings on neutrophils infiltrating CRC are more controversial (Mizuno et al., 2019), as high counts of intratumoural neutrophils correlated with higher pT, pM and stage. Swedish research team from Umea University found that neutrophil infiltration in the tumour front is a favourable prognostic factor in early CRC (Wikberg et al., 2017). These controversies might be explained by the duality of neutrophils, which comprise a tumour-suppressive N1 subpopulation as well as a tumour-supportive N2 population (Mizuno et al., 2019).

Changes in TAMs have been widely studied in CRC. In a study with 81 CRC cases, the researchers evaluated TAM relationships with EMT markers (E-cadherin and vimentin). TAM surface antigens CD68 and CD163 were mainly expressed at the tumour invasive front and stroma, with no to weak expression in the tumour nest. Near the tumour invasive front, high CD163 expression was associated with low E-cadherin and high vimentin expression, indicating EMT process. The authors performed univariate and multivariate analyses and showed that CD163 expression at the invasive front was an independent prognostic factor associated with poor relapse free survival (RFS) (HR 2.414, 95 % CI 1.016–4.523, p = 0.045) and overall survival (HR 3.234, 95 % CI 1.176–8.889, p = 0.023) (Wei et al., 2019).

CD8+ T cells have been known to have an effect on CRC patient survival for decades (Naito et al., 1998), and their presence within different malignancies has been associated with a better prognosis (Lalos et al., 2021). In CRC, a low CD8+ T cell density has been associated with infiltrative tumour border, which is one of the indicators for poor prognosis in CRC (Lalos et al., 2021). There were significant differences between the peritumoural CD8+ T cell count and pT ($p = 0.03$) and pN ($p = 0.05$). CRC with a higher pT in the peritumoural tissue had a lower CD8+ T cell count compared with CRC with low pT: 121 (95 % CI 95.7–146.9) in pT4 and 198.5 (95 % CI 82.6–314.4) in pT1. In relation to pN, there was a significant difference in the peritumoural CD8+ T cell count ($p = 0.05$). Interestingly, the peritumoural CD8+ T cell count showed significant differences in terms of perineural ($p = 0.02$) and intraneural ($p = 0.02$) invasion. Lea et al. reported very similar results: Perineural invasion was associated with a low Immunoscore ($p = 0.008$), but lymphatic invasion had no significant association with its changes ($p = 0.10$) (Lea et al., 2021).

The current CRC treatment involves mostly surgery and chemotherapy and / or radiation therapy (Shinagawa et al., 2017). However, as in other malignancies the role of immunotherapy has expanded and has produced good results (Bayraktar, Batoo, Okuno, & Glück, 2019; Lugowska, Teterycz, & Rutkowski, 2018). As most of the metastatic CRC cases are MSS and the possibilities of immunotherapy are still limited in such tumours, researchers have advised intensifying research of the complex tumour microenvironment in association with the microsatellite status (Kather, Halama, & Jaeger, 2018). A recent study published by researchers from France suggests that medication combination of atezolizumab and tiragolumab could restore T cell function in microsatellite-stable CRC (Thibaudin et al., 2022).

3.3 Epithelial mesenchymal transition

EMT is one of the key events in CRC pathogenesis that leads to tumour invasion and metastatic spread (Grigore, Jolly, Jia, Farach-Carson, & Levine, 2016). In CRC, EMT properties such as cytoskeletal deformability and motility and co-expression of EMT markers are evident in small cell clusters known as tumour buds (Grigore et al., 2016). Recently, Sato et al. analysed 32 CRC cases displaying tumour budding. They showed that cancer buds expressed various amounts of LGR5 and PD-L1 and suggested that patients with PD-L1-negative tumour buds should receive a different treatment that targets the CSC marker LGR5 (Sato et al., 2021). Thus, tumour budding could be closely associated with stemness. This linkage is not limited to CRC but is likely to reflect a general feature of carcinogenesis. One of the CRC stem cell property markers is CD44, and its upregulation within CRC tissues is associated with aggressive tumour behaviour (Mohamed et al., 2019) as well as resistance to chemotherapy (Pothuraju et al., 2020).

In the current study, there were **CD44** expression changes in relation to CRC morphology, as its overexpression was seen in mucinous CRC (2.00, 95 % CI 1.61–2.38) compared with adenocarcinomas (1.28, 95 % CI 1.15–1.41). On the contrary, studies have shown loss of CD44 expression in mucinous carcinomas (Ismaiel et al., 2016). In the current study, CD44 overexpression was associated with right-sided tumours ($p = 0.002$), but there were no significant differences in CD44 expression in terms of pT, pG, manifestations of invasive growth, and inflammation. Interestingly, there was significantly higher CD44 expression in pN0 tumours. Such a phenomenon has also been described in mucinous ovarian carcinomas, as borderline tumours have been found to express higher levels of CD44 than invasive carcinomas (Matuura et al., 2018). In CRC, this phenomenon could possibly be explained by CD44

isoform switch, as described previously (Mashita et al., 2014; Z. Wang et al., 2019).

E-cadherin expression also showed significant differences according to tumour morphology ($p = 0.001$), with a higher expression score in colorectal adenocarcinoma (1.91, 95 % CI 1.83–1.98) than in mucinous carcinoma (1.48, 95 % CI 1.23–1.73). Nevertheless, downregulation of E-cadherin was associated with lower differentiation ($p = 0.001$), as has also been described in other studies (Mogoantă et al., 2014; Sayar et al., 2015). There was lower E-cadherin expression in right-sided compared with left-sided tumours ($p = 0.02$). In the current study, E-cadherin expression did not differ according to pT ($p = 0.07$), pN ($p = 0.16$), and invasive growth ($p > 0.05$). Other studies have indicated a role for E-cadherin expression loss in tumour progression, as it is considered to be marker for extranodal tumour extension, that is highly associated with vascular invasion and high pN stage in pT3 CRC (Kim et al., 2019).

β-catenin expression showed significant association between higher pT and lower β-catenin expression ($p = 0.007$). Previous researches show controversial results regarding associations of β-catenin expression and pT and pG. Lee et al. in their research showed that β-catenin expression has no difference according to pT, as more advanced tumours (pT3 and pT4) showed β-catenin expression in the same percentage of cases , as in pT1 and pT2 ($p = 0.07$), however within their study low grade CRC was more likely showing positive b-catenin expression (62.5 % of cases), than in high grade CRC, where β-catenin expression was observed in 38.9 % of cases (K. S. Lee et al., 2016). In difference in study by Gao et al. reduced membranous β-catenin expression levels at the tumour centre were identified to be significantly associated with the occurrence of lymph node metastasis ($P = 0.002$) and the TNM stage ($P = 0.002$) (Gao, Lu, Wang, Han, & Guo, 2014).

N-cadherin is one of the components of the EMT, and its overexpression has been associated with a worse prognosis in a various tumours (Noh et al., 2017). N-cadherin expression differed significantly with regard to pT ($p = 0.03$): Higher N-cadherin expression was associated with pT3 tumours (1.97, 95 % CI 1.71–2.23), although slightly lower N-cadherin expression was observed for pT4 tumours (1.45, 95 % CI 1.12–1.78). In previous studies that have also used semi-quantitative evaluation of N-cadherin, researchers have noticed several significant associations in N-cadherin expression in terms of tumour differentiation, tumour size, invasion, and the presence of metastasis (Xuebing Yan et al., 2015). Interestingly, in the current study N-cadherin expression was significantly different in tumours with sCRC, showing lower expression in these tumours. N-cadherin downregulation is mostly associated with reduced migration and invasion in a various malignancies (K. Li, He, Lin, Wang, & Fan, 2010; X. F. Zhang, Zhang, Chang, Wu, & Guo, 2018). Future research could evaluate N-cadherin expression in sCRC.

3.4 Interactions of EMT and inflammation

Researchers have described relationships between EMT markers. There is extensive evidence on how downregulation of E-cadherin leads to upregulation of N-cadherin (Cao, Wang, & Leng, 2019; Jang, Choi, & Gu, 2021), and how levels of CD44 and β-catenin differ at various stages of CRC (Iseki et al., 2017; Masaki et al., 2001; Qu et al., 2017). However, the relationships between EMT markers and different degrees of inflammation has not been widely studied.

Various cell studies have demonstrated the role of local inflammation in potential tumour spread. Indeed, cytokines such as IL-1β, IL-6, and TNFα stimulate CRC cell adhesion to the mesothelium (van Grevenstein et al., 2007), indicating their potential role in CRC spread. This theory was overlooked by scientists from Poland, who in *in vitro* and *in vivo* mouse studies showed that

colorectal SW480 cell proliferation and invasion was stimulated by IL-6 and development of EMT by TGF-β1 released from mesothelial cell lines (Mikuła-Pietrasik J. et al., 2015). They also studied the effects of the EMT-driving TFs SMAD2/3 and Snail1, as inhibition of these TFs significantly decreased transformation of CRC cells into mesenchymal phenotype with decreased expression of E-cadherin and increased vimentin expression (Mikuła-Pietrasik J. et al., 2015).

In the current study, there was a significant albeit weak correlation between low-grade inflammation and β-catenin and E-cadherin expression ($r/rs = 0.290$, $p = 0.03$). There was also a significant, moderate correlation between β-catenin and N-cadherin expression ($r/rs = 0.491$, $p = 0.00$), but there were no other significant correlations between marker expression and degree of inflammation. These results could indicate the potential role of inflammation, but local inflammation within CRC might not be the only thing driving CRC development, as systemic inflammation has tumour-driving potential in several cancers, including CRC (Chen et al., 2017).

Cell subpopulation analysis in the current study revealed a significant difference in β-catenin expression in terms of the neutrophil count: A high neutrophil count was associated with a higher β-catenin expression ($p = 0.001$). Previous research has shown that one of the cytokines responsible for neutrophil recruitment (IL-37) is responsible for suppressing β-catenin expression within CRC (Xiaofei Yan, Zhao, & Zhang, 2017), showing the possible anti-tumoural activity of neutrophils. Although the current study did not show differences in β-catenin expression and the lymphocyte count ($p = 0.68$), the presence of neutrophils could be explained by a lymphocyte-related reaction as they promote neutrophil activation in a various directions (Oberg, Wesch, Kalyan, & Kabelitz, 2019). Upregulation of β-catenin in the presence of neutrophils supports previous

research, where high a intratumoural neutrophil count in CRC was associated with poor prognosis (Rao et al., 2012).

Interestingly, analysis of inflammatory cell subpopulations showed significant results in terms of upregulation of E-cadherin and a high eosinophil density ($p = 0.007$). A protective association ($p = 0.003$) has been reported between increased blood count of eosinophils and decreased risk of CRC, with HRs of 1.0, 0.70 (95 % CI 0.50–0.98), and 0.58 (95 % CI 0.40–0.83) across the tertiles of the absolute eosinophil count (Prizment, Anderson, Visvanathan, & Folsom, 2011). A protective role has also been ascribed to stromal eosinophils in CRC (A. E. Prizment et al., 2016). Thus, E-cadherin upregulation is associated with beneficial tumour features, possibly including recruitment of eosinophils as one of the mechanisms.

Inflammation drives EMT via a complex mechanism, as E-cadherin overexpression in macrophages could indicate potential immunosuppression of some subpopulation of macrophages. Researchers showed that alternatively activated macrophages (AAMs) expressing E-cadherin on their surface have an immune-suppressing role, as in AAMs lower production of inflammatory cytokines was observed (Van den Bossche et al., 2015). However, another mouse study showed that E-cadherin levels could be a potential indicator for inflammation severity, as mice with acute pancreatitis had elevated E-cadherin expression and statistical analysis showed a significant association with severe acute pancreatitis (Yuan et al., 2015).

These results indicate complex mechanisms involved in EMT: The network leading to EMT could be more complicated, and inflammation could play a significant role in this process as well.

3.5 Mismatch repair proteins

Detection of MMR gene aberrations (*MSH2*, *MSH6*, *PMS2*, and *MLH1*) is crucial in CRC, as loss of MMR protein production is an indicator for MSI in carcinomas. In the era of personalized medicine, precise diagnosis is crucial, as MSI tumours have shown resistance to chemotherapy (Aggarwal, Quaglia, McPhail, & Monahan, 2022; Jo & Carethers, 2006). MLH1 and MSH2 IHC has acceptable sensitivity and specificity for high-MSI status: 92.3 % and 100 %, respectively (Lindor et al., 2002). Therefore, this approach has been applied in other studies (Amira et al., 2014; Lanza et al., 2002), and IHC detection of MMR proteins is considered to be an appropriate alternative to molecular testing (Raffone et al., 2020). Moreover, IHC-based detection of MMR proteins is more cost-effective and not as time-consuming as other methods (Bai et al., 2019; Pérez-Carbonell et al., 2012).

There were significant differences in MSH6 ($p = 0.03$) and PMS2 ($p = 0.002$) expression in relation to CRC histogenesis, as signet ring cell carcinoma showed markedly lower expression of MSH6 (0.07 [0.0–0.39]) and PMS2 (0.02 [0.0–0.21]) compared with adenocarcinoma (MSH6: 1.84 [1.69–1.98]; PMS2: 1.54 [1.39–1.70]). Previous studies have also reported loss of MMR protein expression via IHC in poorly differentiated CRC (S.-M. Wang et al., 2019).

Although in the current research there were no significant differences in MMR protein expression in relation to the degree of inflammation, MSH2, MSH6, PMS2, and MLH1 expression was different according to tumour location, with significantly higher expression in left-sided tumours. MSH2 and PMS2 expression was different depending on pG, showing significantly lower expression within high- and low-grade tumours, while in the case of moderate differentiation the levels of both MMR proteins were higher. There were no differences in MMR protein expression based on local structure involvement

except in case of intraneural invasion and MSH6 expression, as absence of invasion was characterized by higher MSH6 expression.

Researchers have also shown differences in MMR protein expression according to tumour location and pG. Wang et al. found that MLH1/MSH2-negative CRC occurred more frequently in the right than in the left colon (27.88 % vs. 17.86 %, p = 0.029), and MMR negativity was associated with poor differentiation and mucin production (S.-M. Wang et al., 2019). However, changes in MLH1/MSH2 expression were not associated with age, gender, tumour stage, tumour size, lymphocytic infiltration, or circumscribed margin (P > 0.05) (S.-M. Wang et al., 2019). Recently, researchers noted that about 25 % of patients with sCRC have intratumoural changes in MMR protein expression, suggesting that molecular mechanisms affecting development of sCRC varies and detection of changes in MMR expression is necessary in treatment decision (Vyas et al., 2021). In the current study, differences in MMR protein expression did not differ in sCRC cases compared with cases without sCRC (p > 0.05). However, further investigation is needed given the small number of sCRC cases in the current study.

Certain MMR proteins showed associations with the molecular characteristics of CRC. A higher mean E-cadherin score was significantly associated with the presence of MSH2 (p = 0.008) and PMS2 (p = 0.014), while lower CD44 expression was associated with PMS2 (p = 0.05). There were no differences with regard to β-catenin and N-cadherin expression and MMR proteins. Only a few studies have analysed MMR status and molecular characteristics in CRC. A recent study demonstrated that only MMR-proficient CRC that lacked evidence of differentiation totally lost E-cadherin expression (Perna et al., 2021).

In the current study, the expression of MMR proteins did not differ depending on the intensity of inflammation (low and high, based on the Klintrup-Mäkinen score). There were no significant differences in MMR protein expression among the inflammatory cell subpopulation, except higher MLH1 expression in cases with a higher neutrophil density ($p = 0.02$). This is different from a recent study in which patients with neutrophil-rich CRC show higher rates of MMR-deficient CRC ($p < 0.01$). Nevertheless, those authors showed that the neutrophil count does not affect 5-year survival rates, as MMR-proficient and MMR-deficient CRC showed similar survival rates in neutrophil rich carcinomas (Rottmann et al., 2021).

In the current study, MSH6 levels showed a significant correlation with the peritumoural CD8+ T cell count ($r/rs = 0.203$, $p = 0.031$), indicating its possible role toward immune mechanism activation. However, there were no significant differences when analyzing low MMR versus high MMR protein expression, as well as presence / absence of MMR proteins and the peritumoural CD8+ T cell count. In a previous study, researchers described a correlation between CD8+ T cells and MSH2 expression in lung adenocarcinoma: High MSH2 expression correlated strongly with increased PD-L1 expression and CD8+ T cell infiltration. This finding led the authors to think about the potential for immunotherapy as detection of MMR proteins via ICH is relatively easy and fast (Jia, Yao, Yang, & Chi, 2020). Nevertheless, there are other factors that could affect the CD8+ T cell count, as in CRC immature desmoplastic stromal reaction have been associated with lower CD8+ T cell and macrophage counts, as well as normal MMR protein expression (Ueno et al., 2014).

Overall this study shows complex network connecting MMR proteins, inflammation and EMT and indicates need for further studies.

Conclusions

1. This study overall shows high distribution of locally advanced CRC in Latvia, presenting as pT4 and pN+.
2. Although the overall incidence of synchronous adenomas corresponds to other studies, villous adenomas were more frequent. The distribution of sCRC was higher in younger patients, indicating a possible role of inherited mutations within its development.
3. High-grade inflammation significantly less frequently features specific manifestations of the higher invasive capacity of the tumour, including perineural and intraneural growth and invasion into lymphatic vessels, veins, and arteries. Thus, high-grade peritumoural inflammation is associated with beneficial morphologic features of CRC and is not secondary to tissue damage and necrosis.
4. Low peritumourous and intratumourous lymphocytic, neutrophil and eosinophil density is associated with local structure involvement, and absence of CLR is associated with more advanced tumours in their local spread.
5. In CRC EMT is connected to histogenesis and differentiation of tumour: it is more common in low differentiated adenocarcinomas and tumours with non-adenocarcinoma morphology, as E-cadherin expression significantly decreases. EMT has a role in a tumour progression and it could partially be affected by inflammation.
6. CD44 expression depends on histogenesis of tumour; however, it does not depend on differentiation of tumour. Lower expression of CD44 within CRC could be a marker for lymphogenous metastasis.
7. β-catenin could be used as an indicator for advanced CRC, as its levels significantly increases in tumours with higher pT. Levels of β-catenin are associated with immune response.

8. N-cadherin levels significantly decreases and E-cadherin expression significantly increases in tumours with high amounts of eosinophils, indicating protective role of these immune cells. However, lower N-cadherin expression were seen in CRC cases with sCRC.
9. MMR protein expression significantly decreases in non-adenocarcinomas and right side tumours, however their expression is not significantly different in tumours with and without local structure involvement as well as in tumours with high- and low-grade inflammation. MLH1 protein expression significantly differs in tumours according to neutrophilic leucocyte infiltration, potentially indicating molecular mechanism role in anti-tumour immunity.
10. MMR status potentially affects EMT, as in tumours with low MMR protein expression significantly decreases amounts of E-cadherin and increases CD44 expression.

Practical recommendations

1. The histopathology report for each CRC case should include evaluation of inflammation based on the Klintrup-Mäkinen score.
2. Immunohistochemical investigation should be performed in all CRC cases to evaluate stemness (CD44) and MMR protein (MSH2, MSH6, PMS2, MLH1) expression.

Publications and reports on topics of the Doctoral Thesis

Publications

1. **Briede**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2020. The Association Between Inflammation, Epithelial Mesenchymal Transition and Stemness in Colorectal Carcinoma. *J Inflamm Res*. Jan 8; 13:15–34. doi: 10.2147/JIR.S224441.

2. **Briede**, **I**., Balodis, D., Gardovskis, J., Strumfa, I. 2021. Stemness, Inflammation and Epithelial-Mesenchymal Transition in Colorectal Carcinoma: The Intricate Network. *Int J Mol Sci*. Nov 29; 22(23):12891. doi: 10.3390/ijms222312891.

3. **Driķe**, **I**., Strumfa, I., Kolomencikova, L., Vasko, E., Vanags, A., Gardovskis, J. 2014. Colorectal leiomyosarcoma- a rare tumour in GIST era. *Acta Chirurgica Latviensis*. 14/2: 36–39.

4. Vasko, E., Vanags, A., Strumfa, I., Bogdanova, T., **Drike**, **I**., Gardovskis, J. 2014. Malignant neighbours in liver: co-occurence of metastatic colorectal and hepatocellular carcinomas. *Acta Chirurgica Latviensis*. 14/2: 49–51.

5. **Driķe**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2014. Frequency of morphologic prognostic factors in surgically treated colorectal cancer. *Acta Chirurgica Latviensis*. 14/1: 3–10.

Reports and theses at international congresses and conferences

1. **Briede**, **I**., Strumfa, I., Konopecka, V., Ratniece, M., Vanags, A., Gardovskis, J. 2021. Inflammation – the microenvironment for tumor progression and stem cell differentiation in colorectal carcinoma. Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice": Abstracts, 24–26 March, 461.

2. Cukure, F., Cipkina, S., **Driķe**, **I**., Strumfa, I., Gardovskis, J. 2019. Tumor volume association with the systemic inflammatory reaction in patients with surgically treated colorectal carcinoma. Abstract Volume, World Congress of Surgery, 11.–15.08.2019. Krakow, Poland.

3. **Drike**, **I**., Cukure, F., Cipkina, S., Strumfa, I., Gardovskis, J. 2019. C- reactive protein and other SIR parameters in relation to lymph node yield in colorectal carcinoma. RSU International research conference 2019 – Knowledge for use in practice. April 1st-3rd, 2019, Riga Latvia.

4. **Drike**, **I**., Cipkina, S., Cukure, F., Strumfa, I., Gardovskis, J. 2019. Interaction between local and systemic inflammatory response in colorectal carcinoma: two faces of Janus. RSU International research conference 2019 – Knowledge for use in practice. April 1st–3rd, 2019, Riga Latvia.

5. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2017. Mismatch repair proteins and epithelial mesenchymal transition in colorectal cancer. 29th Congress of the ESP Amsterdam, Netherlands 02.09.–06.09.2017. Theses book: Virchows Arch. The European Journal of Pathology. Springer, 2017; 471 (1), 179.

6. Rumba, R., Vanags, A., **Drike**, **I**., Cukure, F., Cipkina, S., Gardovskis, J., Strumfa, I. 2017. Systemic inflammatory reaction in surgically treated colorectal cancer. Abstract Volume, World Congress of Surgery, 13.–17.08.2017. Basel, Switzerland.

7. Rumba, R., **Drike**, **I**., Vanags, A., Strumfa, I., Gardovskis, J. 2017. Epithelial mesenchymal transformation and peritumorous inflammation in surgically treated colorectal cancer. Abstract Volume, World Congress of Surgery, 13.–17.08.2017. Basel, Switzerland.

8. **Drike**, **I**., Strumfa, I., Rumba, R., Vanags, A., Gardovskis, J. 2017. Landscape of CD44expression in colorectal carcinoma by tumour and patient's characteristics. Poster session abstracts. 8th Mildred Scheel cancer conference, 2017, 149.

9. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2016. Intensity of inflammation and lymphoid follicle density in relation to the invasive properties of colorectal cancer. Abstract Book Posters. 8th European Multidisciplinary colorectal cancer congress (EMCCC), 2016, 62, 41.

10. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2016. Calretinin use for serosal invasion detection in case of colorectal cancer. Virchows Arch. *The European Journal of Pathology*. Springer. 465 (1), 313.

11. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2016. Modified Klintrup-Makinen inflammation score in relation to invasive properties of colorectal cancer. International symposium *Targets of immunotherapy of chronic viral infections and cancer*. Riga, Latvia, May 24–26.

12. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2016. p53 protein expression and its correlation with cell proliferation in colorectal cancer. International symposium *Targets of immunotherapy of chronic viral infections and cancer*. Riga, Latvia, May 24–26.

13. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2015. Tumour necrosis in colorectal cancer: an association with the invasive potential. The 8th Baltic Morphology scientific conference. Vilnius, Lithuania, 12.–14.11.2015., Abstract Book, 87.

14. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2015. E-cadherin as a prognostic factor in locally advanced colorectal cancer. The 8th Baltic Morphology scientific conference. Vilnius, Lithuania, 12.–14.11.2015., Abstract Book, 86.

15. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2015. Tumour volume: lack of biological significance in colorectal cancer. Abstract Book of the 8th Congress of the Baltic Association of Surgeons. Tallin, Estonia, 10.–12.09.2015., 67.

16. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2015. Regional lymph node status in synchronous colorectal carcinoma. Abstract Book of the 8th Congress of the Baltic Association of Surgeons. Tallin, Estonia, 10.–12.09.2015., 66.

17. Vasko, E., Vanags, A., Strumfa, I., **Drike**, **I**., Gardovskis, J. 2015. Immunohistochemical factors associated with metastatic spread of surgically treated colorectal cancer. 46th World Congress of Surgery, Bangkok, Thailand, 23.–27.08.2015., Abstract Book, 441.

Theses at local congresses and conferences:

1. **Drike**, **I**., Vanags, A., Strumfa, I., Gardovskis, J. 2018. Inflammatory cell spectrum in colorectal carcinoma, its relation to tumour invasion [Iekaisuma šūnu spektrs kolorektālas karcinomas audos saistībā ar audzēja invāziju] Abstracts of the 17th scientific conference, Riga Stradins University (22nd–23rd April, 2018), 176.

2. **Drike**, **I**., Vanags, A., Strumfa, I., Gardovskis, J. 2018. Amount of lymphnodes in colorectal carcinoma in different intensity inflammation [Apzarņa limfmezglu skaits kolorektālās karcinomās ar dažādas intensitātes iekaisumu]. Abstracts of the 17th scientific conference, Riga Stradins University (22nd–23rd April, 2018), 178.

3. Rumba, R., Vanags, A., Cipkina, S., Cukure, F., **Drike**, **I**., Gardovskis, J., Strumfa, I. 2018. Systemic inflammatory reaction and its relation to surgically treated colorectal carcinoma morphology [Sistēmiska iekaisuma reakcijas saistība ar ķirurģiski ārstētas kolorektālas karcinomas lokālo morfoloģisko ainu] Abstracts of the 17th scientific conference, Riga Stradins University (22nd–23rd April, 2018), 174.

4. **Drike**, **I**., Strumfa, I., Rumba, R., Vanags, A., Gardovskis, J. 2017. Manifestation of epithelial mesenchymal transition in colorectal cancer [Epiteliāli mezenhimālās transformācijas izpausmes kolorektālā vēzī]. Abstracts of the 16th scientific conference, Riga Stradins University (6th–7th April, 2017), 205.

5. **Drike**, **I**., Strumfa, I., Rumba, R., Vanags, A., Gardovskis, J. 2017. CD44 expression in colorectal cancer [CD44 ekspresija kolorektālā vēzī]. Abstracts of the 16th scientific conference, Riga Stradins University (6th–7th April, 2017), 206.

6. Strumfa, I., **Drike**, **I**., Simtniece, Z., Mezale, D., Fridrihsone, I., Štrumfs, B., Abolins, A., Balodis, D., Vanags, A., Gardovskis, J. 2017. Expression of vimentin in adenocarcinoma cells of different origin: insight of epithelial mesenchymal transition [Vimentīna ekspresija dažādas izcelsmes adenokarcinomu šūnās: ieskats epiteliāli mezenhimālajā transformācijā] Abstracts of the 16th scientific conference, Riga Stradins University (6th–7th April, 2017), 221.

7. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2016. Cell adhesion prognostic role in colorectal cancer. [Šūnu adhēzijas prognostiskā nozīme kolorektālā vēzī] // Abstracts of the 15th scientific conference, Riga Stradins University (17th–18th March, 2016), 188.

8. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2016. p53 protein expression in colorectal cancer [p53 proteīna ekspresija kolorektālā vēzī] // Abstracts of the 15th scientific conference, Riga Stradins University (17th–18th March, 2016), 189.

9. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2016. Inflammation intensity and Crohn- like lymphoid reaction significance in colorectal cancer [Iekaisuma intensitātes un Krona slimībai līdzīgās limfoīdās reakcijas nozīme kolorektālā vēzī] // Abstracts of the 15th scientific conference, Riga Stradins University (17th–18th March, 2016), 190.

10. **Drike**, **I**., Strumfa, I., Uzars, A., Zevaka, O., Kolomencikova, L., Vanags, A., Gardovskis, J. 2015. Connection of colorectal tumour morphological spectrum to the patients age and tumour localisation [Kolorektālu audzēju morfoloģiskā spektra saistība ar pacienta vecumu un audzēja lokalizāciju] // Abstracts of the 15th scientific conference, Riga Stradins University (26th–27th March, 2015), 278.

11. **Drike**, **I**., Strumfa, I., Kolomencikova, L., Zevaka, O., Uzars, A., Vanags, A., Gardovskis, J. 2015. Colorectal cancer volume and its connection to the tumour localisation and pT [Kolorektāla vēža tilpums saistībā ar audzēja lokalizāciju un pT] // Abstracts of the 15th scientific conference, Riga Stradins University (26th–27th March, 2015), 279.

12. Strumfa, I., Vasko, E., Vanags, A., **Drike**, **I**., Gardovskis, J. 2015. Molecular factors for metastasis formation in surgically treated colorectal carcinoma [Metastāžu veidošanās molekulārie faktori ķirurģiski ārstētā kolorektālā karcinomā] // Abstracts of the 15th scientific conference, Riga Stradins University (26th–27th March, 2015), 275.

13. Strumfa, I., Vasko, E., Vanags, A., **Drike**, **I**., Gardovskis, J. 2015. Molecular portret of liver surgery: comparement of most frequently resected carcinomas [Aknu ķirurģijas molekulārais portrets: biežāko rezecējamo karcinomu salīdzinājums] // Abstracts of the 15th scientific conference, Riga Stradins University (26th–27th March, 2015), 277.

14. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2014. The morphologic spectrum of surgically treated colorectal cancer [Radikāli operēta kolorektāla vēža morfoloģiskais spektrs] // Abstracts of the 13th scientific conference, Riga Stradins University (10th–11th April, 2014), 295.

15. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2014. Morphologic prognostic factors of surgically treated colorectal cancer [Kolorektāla vēža prognostisko faktoru analīze radikālu operāciju materiālā] // Abstracts of the 13th scientific conference, Riga Stradins University (10th–11th April, 2014), 296.

16. **Drike**, **I**., Strumfa, I., Vanags, A., Gardovskis, J. 2014. Regional lymph node evaluation in surgical materials of colorectal carcinoma [Reģionālo limfmezglu stāvoklis kolorektālas karcinomas gadījumā] // Abstracts of the 13th scientific conference, Riga Stradins University (10th–11th April, 2014), 297.

17. Vilmanis, J., Vanags, A., Strumfa, I., **Drike**, **I**., Simtniece, Z., Vasko, E., Gardovskis, J. 2014. Immunohistochemical algorithm in the differential diagnostics of hepatocellular and colorectal cancer [Imūnhistoķīmiskais algoritms hepatocelulāra vēža un metastātiska kolorektāla vēža diagnostikai] // Abstracts of the 13th scientific conference, Riga Stradins University (10th–11th April, 2014), 289.

18. Vanags, A., Strumfa, I., Abolins, A., Simtniece, Z., **Drike**, **I**., Gardovskis, J. 2014. Mucinous cystadenoma of the appendix: analysis of morphological progression and clinical picture [Aklās zarnas tārpveida piedēkļa mucinoza cistadenoma: morfoloģiskās progresijas un klīniskās ainas analīze] // Abstracts of the 13th scientific conference, Riga Stradins University (10th–11th April, 2014), 282.

References

1. Abotchie, P. N., Vernon, S. W., Du, X. L. 2012. Gender differences in colorectal cancer incidence in the United States, 1975–2006. *Journal of women's health.* 21(4), 393–400. doi: 10.1089/jwh.2011.2992

2. Aggarwal, N., Quaglia, A., McPhail, M. J. W., Monahan, K. J. 2022. Systematic review and meta-analysis of tumour microsatellite-instability status as a predictor of response to fluorouracil-based adjuvant chemotherapy in colorectal cancer. *Int J Colorectal Dis.* 37(1), 35–46. doi: 10.1007/s00384-021-04046-x

3. Amira, A. T., Mouna, T., Ahlem, B., Raoudha, A., Majid, B. H., Amel, H., Nadia, K. 2014. Immunohistochemical expression pattern of MMR protein can specifically identify patients with colorectal cancer microsatellite instability. *Tumour Biol.* 35(7), 6283–6291. doi: 10.1007/s13277-014-1831-2

4. Bai, W., Ma, J., Liu, Y., Liang, J., Wu, Y., Yang, X., Xi, Y. 2019. Screening of MSI detection loci and their heterogeneity in East Asian colorectal cancer patients. *Cancer medicine.* 8(5), 2157–2166. doi: 10.1002/cam4.2111

5. Bayraktar, S., Batoo, S., Okuno, S., Glück, S. 2019. Immunotherapy in breast cancer. *Journal of carcinogenesis.* 18, 2–2. doi: 10.4103/jcar.JCar_2_19

6. Betge, J., Pollheimer, M. J., Lindtner, R. A., Kornprat, P., Schlemmer, A., Rehak, P., Langner, C. 2012. Intramural and extramural vascular invasion in colorectal cancer. *Cancer*. 118(3), 628–638. doi: 10.1002/cncr.26310

7. Bouquot, M., Creavin, B., Goasguen, N., Chafai, N., Tiret, E., André, T., Svrcek, M. 2018. Prognostic value and characteristics of N1c colorectal cancer. *Colorectal Disease.* 20(9), O248–O255. doi: https://doi.org/10.1111/codi.14289

8. Cao, Z. Q., Wang, Z., Leng, P. 2019. Aberrant N-cadherin expression in cancer. *Biomed Pharmacotherap*. 118, 109320. doi: 10.1016/j.biopha.2019.109320

9. Chen, J.-H., Zhai, E.-T., Yuan, Y.-J., Wu, K.-M., Xu, J.-B., Peng, J.-J., Cai, S.-R. 2017. Systemic immune-inflammation index for predicting prognosis of colorectal cancer. *World Journal of Gastroenterology*. 23(34), 6261–6272. doi: 10.3748/wjg.v23.i34.6261

10. De Smedt, L., Lemahieu, J., Palmans, S., Govaere, O., Tousseyn, T., van Cutsem, E., Sagaert, X. 2015. Microsatellite instable vs stable colon carcinomas: analysis of tumour heterogeneity, inflammation and angiogenesis. *Br J Cancer.* 113(3), 500–509. doi: 10.1038/bjc.2015.213

11. Fabregat, I., Malfettone, A., Soukupova, J. 2016. New Insights into the Crossroads between EMT and Stemness in the Context of Cancer. *J Clin Med.* 5(3), 37. doi: 10.3390/jcm5030037

12. Fedyanin, M., Popova, A., Polyanskaya, E., Tjulandin, S. 2017. Role of Stem Cells in Colorectal Cancer Progression and Prognostic and Predictive Characteristics of Stem Cell Markers in Colorectal Cancer. *Curr Stem Cell Res Ther.* 12(1), 19–30. doi: 10.2174/1574888x11666160905092938

13. Galdiero, M. R., Garlanda, C., Jaillon, S., Marone, G., Mantovani, A. 2013. Tumor associated macrophages and neutrophils in tumor progression. *J Cell Physiol.* 228(7), 1404–1412. doi: 10.1002/jcp.24260

14. Gao, Z.-H., Lu, C., Wang, M.-X., Han, Y., Guo, L.-J. 2014. Differential β-catenin expression levels are associated with morphological features and prognosis of colorectal cancer. *Oncol Lett.* 8(5), 2069–2076. doi: 10.3892/ol.2014.2433

15. Ghazi, S., Lindforss, U., Lindberg, G., Berg, E., Lindblom, A., Papadogiannakis, N., Low-Risk Colorectal Cancer Study, G. 2012. Analysis of colorectal cancer morphology in relation to sex, age, location, and family history. *J Gastroenterol.* 47(6), 619–634. doi: 10.1007/s00535-011-0520-9

16. Ghebrial, M., Aktary, M. L., Wang, Q., Spinelli, J. J., Shack, L., Robson, P. J., Kopciuk, K. A. 2021. Predictors of CRC Stage at Diagnosis among Male and Female Adults Participating in a Prospective Cohort Study: Findings from Alberta's Tomorrow Project. *Current oncology (Toronto, Ont.).* 28(6), 4938–4952. doi: 10.3390/curroncol28060414

17. Grigore, A. D., Jolly, M. K., Jia, D., Farach-Carson, M. C., Levine, H. 2016. Tumor Budding: The Name is EMT. Partial EMT. *J Clin Med.* 5(5), 51. doi: 10.3390/jcm5050051

18. Hanahan, D., Coussens, L. M. 2012. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell.* 21(3), 309–322. doi: 10.1016/j.ccr.2012.02.022

19. Harbaum, L., Pollheimer, M. J., Kornprat, P., Lindtner, R. A., Bokemeyer, C., Langner, C. 2015. Peritumoral eosinophils predict recurrence in colorectal cancer. *Mod Pathol.* 28(3), 403–413. doi: 10.1038/modpathol.2014.104

20. Hogan, J., Chang, K. H., Duff, G., Samaha, G., Kelly, N., Burton, M., Coffey, J. C. 2015. Lymphovascular Invasion: A Comprehensive Appraisal in Colon and Rectal Adenocarcinoma. *Diseases of the Colon & Rectum*, 58(6).

21. Hosseini, S., Bananzadeh, A. M., Salek, R., Zare-Bandamiri, M., Kermani, A. T., Mohammadianpanah, M. 2017. Prognostic Significance of Mucinous Histologic Subtype on Oncologic Outcomes in Patients With Colorectal Cancer. *Ann Coloproctol.* 33(2), 57–63. doi: 10.3393/ac.2017.33.2.57

22. Iseki, Y., Shibutani, M., Maeda, K., Nagahara, H., Ikeya, T., Hirakawa, K. 2017. Significance of E-cadherin and CD44 expression in patients with unresectable metastatic colorectal cancer. *Oncol Lett.* 14(1), 1025–1034. doi: 10.3892/ol.2017.6269

23. Ismaiel, N. E. H. S., Sharaf, W. M., Helmy, D. O., Zaki, M. M., Badawi, M. A., Soliman, A. S. A. 2016. Detection of Cancer Stem Cells in Colorectal Cancer: Histopathological and Immunohistochemical Study. *Open access Macedonian journal of medical sciences*. 4(4), 543–547. doi: 10.3889/oamjms.2016.126

24. Jakubowska, K., Koda, M., Grudzińska, M., Lomperta, K., Famulski, W. 2021. Tumor-infiltrating lymphocytes in tissue material combined with systemic lymphocyte inflammation in patients with colorectal cancer. *Mol Clin Oncol*. 14(5), 97–97. doi: 10.3892/mco.2021.2259

25. Jang, N. R., Choi, J., & Gu, M. J. 2021. Aberrant Expression of E-cadherin, N-cadherin, and P-cadherin in Clear Cell Renal Cell Carcinoma: Association with Adverse Clinicopathologic Factors and Poor Prognosis. *Appl Immunohistochem Mol Morphol*. 29(3), 223–230. doi: 10.1097/PAI.0000000000000861

26. Jia, M., Yao, L., Yang, Q., Chi, T. 2020. Association of MSH2 Expression With Tumor Mutational Burden and the Immune Microenvironment in Lung Adenocarcinoma. *Front Oncol.* 10, 168–168. doi: 10.3389/fonc.2020.00168

27. Jiang, Y., You, K., Qiu, X., Bi, Z., Mo, H., Li, L., Liu, Y. 2018. Tumor volume predicts local recurrence in early rectal cancer treated with radical resection: A retrospective observational study of 270 patients. *International Journal of Surgery*. 49, 68–73. doi: https://doi.org/10.1016/j.ijsu.2017.11.052

28. Jo, W.-S., Carethers, J. M. 2006. Chemotherapeutic implications in microsatellite unstable colorectal cancer. *Cancer biomarkers : section A of Disease markers*. 2(1–2), 51–60. doi: 10.3233/cbm-2006-21-206

29. Kather, J. N., Halama, N., Jaeger, D. 2018. Genomics and emerging biomarkers for immunotherapy of colorectal cancer. *Semin Cancer Biol*. 52, 189–197. doi: 10.1016/j.semcancer.2018.02.010

30. Keerthivasan, S., Aghajani, K., Dose, M., Molinero, L., Khan, M. W., Venkateswaran, V., Gounari, F. 2014. β-Catenin promotes colitis and colon cancer through imprinting of proinflammatory properties in T cells. *Science translational medicine*. 6(225), 225ra228–225ra228. doi: 10.1126/scitranslmed.3007607

31. Kim, H., Shin, S., Kim, Y., Bang, S., Park, S., Jee, S., Paik, S. 2019. The clinicopathologic significance of extranodal tumor extension in locally advanced (pT3) colorectal adenocarcinoma and its association with the loss of E-cadherin expression. *International journal of clinical and experimental pathology*. 12(9), 3417–3425.

32. Klintrup, K., Mäkinen, J. M., Kauppila, S., Väre, P. O., Melkko, J., Tuominen, H., Mäkinen, M. J. 2005. Inflammation and prognosis in colorectal cancer. *European Journal of Cancer*. 41(17), 2645–2654. doi: 10.1016/j.ejca.2005.07.017

33. Kojima, M., Shimazaki, H., Iwaya, K., Kage, M., Akiba, J., Ohkura, Y., Ochiai, A. 2013. Pathological diagnostic criterion of blood and lymphatic vessel invasion in colorectal cancer: a framework for developing an objective pathological diagnostic system using the Delphi method, from the Pathology Working Group of the Japanese Society for Cancer of the Colon and Rectum. *J Clin Pathol.* 66(7), 551–558. doi: 10.1136/jclinpath-2012-201076

34. Koper-Lenkiewicz, O. M., Dymicka-Piekarska, V., Milewska, A. J., Zińczuk, J., Kamińska, J. 2021. The Relationship between Inflammation Markers (CRP, IL-6, sCD40L) and Colorectal Cancer Stage, Grade, Size and Location. *Diagnostics (Basel, Switzerland).* 11(8), 1382. doi: 10.3390/diagnostics11081382

35. Kuan, T.-C., Chang, S.-C., Lin, J.-K., Lin, T.-C., Yang, S.-H., Jiang, J.-K., Huang, S.-C. 2019. Prognosticators of Long-Term Outcomes of TNM Stage II Colorectal Cancer: Molecular Patterns or Clinicopathological Features. *World Journal of Surgery.* 43(12), 3207–3215. doi: 10.1007/s00268-019-05158-w

36. Kwak, H. D., Ju, J. K., Lee, S. Y., Kim, C. H., Kim, Y. J., Kim, H. R. 2021. Comparison of Right-side and Left-side Colon Cancers Following Laparoscopic Radical Lymphadenectomy. *Journal of Investigative Surgery.* 34(2), 142–147. doi: 10.1080/08941939.2019.1608334

37. Lalos, A., Tülek, A., Tosti, N., Mechera, R., Wilhelm, A., Soysal, S., Droeser, R. A. 2021. Prognostic significance of CD8+ T-cells density in stage III colorectal cancer depends on SDF-1 expression. *Scientific reports.* 11(1), 775. doi: 10.1038/s41598-020-80382-2

38. Lanza, G., Gafà, R., Maestri, I., Santini, A., Matteuzzi, M., Cavazzini, L. 2002. Immunohistochemical Pattern of MLH1/MSH2 Expression Is Related to Clinical and Pathological Features in Colorectal Adenocarcinomas with Microsatellite Instability. *Modern Pathology.* 15(7), 741–749. doi: 10.1097/01.MP.0000018979.68686.B2

39. Lea, D., Watson, M., Skaland, I., Hagland, H. R., Lillesand, M., Gudlaugsson, E., Søreide, K. 2021. A template to quantify the location and density of CD3 + and CD8 + tumor-infiltrating lymphocytes in colon cancer by digital pathology on whole slides for an objective, standardized immune score assessment. *Cancer immunology, immunotherapy : CII.* 70(7), 2049–2057. doi: 10.1007/s00262-020-02834-y

40. Lee, B. C., Cs, Y., J, K., Jl, L., Cw, K., Ys, Y., Kim, J. C. 2017. Clinicopathological features and surgical options for synchronous colorectal cancer. *Medicine (Baltimore).* 96(9), e6224. doi: 10.1097/MD.0000000000006224

41. Lee, K. S., Kwak, Y., Nam, K. H., Kim, D.-W., Kang, S.-B., Choe, G., Lee, H. S. 2016. Favorable prognosis in colorectal cancer patients with co-expression of c-MYC and ß-catenin. *BMC Cancer.* 16(1), 730–730. doi: 10.1186/s12885-016-2770-7

42. Li, K., He, W., Lin, N., Wang, X., Fan, Q.-X. 2010. Downregulation of N-cadherin Expression Inhibits Invasiveness, Arrests Cell Cycle and Induces Cell Apoptosis in Esophageal Squamous Cell Carcinoma. *Cancer Investigation.* 28(5), 479–486. doi: 10.3109/07357900903476745

43. Li, S., Zhu, K., Yu, W., Wang, Y., Wang, T., Guo, S., Guo, J. 2019. Synchronous Neoplastic Lesions In Referred Patients With Colorectal Cancer: A Retrospective Cohort Study. *Cancer Management and Research*. 11, 9951–9959. doi: 10.2147/CMAR.S229376

44. Li, Z.-P., Liu, X.-Y., Kao, X.-M., Chen, Y.-T., Han, S.-Q., Huang, M.-X., Chu, X.-Y. 2020. Clinicopathological characteristics and prognosis of colorectal mucinous adenocarcinoma and nonmucinous adenocarcinoma: a surveillance, epidemiology, and end results (SEER) population-based study. *Annals of translational medicine*. 8(5), 205–205. doi: 10.21037/atm.2020.01.52

45. Liang, S.-B., Chen, L.-S., Yang, X.-L., Chen, D.-M., Wang, D.-H., Cui, C.-Y., Xu, X.-Y. 2021. Influence of tumor necrosis on treatment sensitivity and long-term survival in nasopharyngeal carcinoma. *Radiotherapy and Oncology.* 155, 219–225. doi: 10.1016/j.radonc.2020.11.011

46. Lim, D. R., Kuk, J. K., Kim, T., Shin, E. J. 2017. Comparison of oncological outcomes of right-sided colon cancer versus left-sided colon cancer after curative resection: Which side is better outcome? *Medicine.* 96(42), e8241–e8241. doi: 10.1097/MD.0000000000008241

47. Lindor, N. M., Burgart, L. J., Leontovich, O., Goldberg, R. M., Cunningham, J. M., Sargent, D. J., Thibodeau, S. N. 2002. Immunohistochemistry Versus Microsatellite Instability Testing in Phenotyping Colorectal Tumors. *Journal of Clinical Oncology.* 20(4), 1043–1048. doi: 10.1200/JCO.2002.20.4.1043

48. Lugowska, I., Teterycz, P., Rutkowski, P. 2018. Immunotherapy of melanoma. *Contemporary oncology (Poznan, Poland).* 22(1A), 61–67. doi: 10.5114/wo.2018.73889

49. Mangone, L., Pinto, C., Mancuso, P., Ottone, M., Bisceglia, I., Chiaranda, G., Rossi, P. G. 2021. Colon cancer survival differs from right side to left side and lymph node harvest number matter. *BMC Public Health.* 21(1), 906–906. doi: 10.1186/s12889-021-10746-4

50. Masaki, T., Goto, A., Sugiyama, M., Matsuoka, H., Abe, N., Sakamoto, A., Atomi, Y. 2001. Possible contribution of CD44 variant 6 and nuclear β-catenin expression to the formation of budding tumor cells in patients with T1 colorectal carcinoma. *Cancer.* 92(10), 2539–2546. doi: https://doi.org/10.1002/1097-0142(20011115)92:10<2539::AID-CNCR1605>3.0.CO;2-I

51. Mashita, N., Yamada, S., Nakayama, G., Tanaka, C., Iwata, N., Kanda, M., Kodera, Y. 2014. Epithelial to mesenchymal transition might be induced via CD44 isoform switching in colorectal cancer. *J Surg Oncol.* 110(6), 745–751. doi: 10.1002/jso.23705

52. Matuura, H., Miyamoto, M., Takano, M., Soyama, H., Aoyama, T., Yoshikawa, T., Furuya, K. 2018. Low Expression of CD44 Is an Independent Factor of Poor Prognosis in Ovarian Mucinous Carcinoma. *Anticancer Research.* 38(2), 717.

53. McCarthy, A. J., Capo-Chichi, J.-M., Spence, T., Grenier, S., Stockley, T., Kamel-Reid, S., Chetty, R. 2019. Heterogenous loss of mismatch repair (MMR) protein expression: a challenge for immunohistochemical interpretation and microsatellite instability (MSI) evaluation. *The journal of pathology. Clinical research.* 5(2), 115–129. doi: 10.1002/cjp2.120

54. Mikuła-Pietrasik, J., Sosińska, P., Maksin, K., Kucińska, M.G., Piotrowska, H., Murias, M., K.siazek, K. (2015). Colorectal cancer-promoting activity of the senescent peritoneal mesothelium. *Oncotarget.* 6(30), 29178–29195. doi: 10.18632/oncotarget.4932

55. Mizuno, R., Kawada, K., Itatani, Y., Ogawa, R., Kiyasu, Y., Sakai, Y. 2019. The Role of Tumor-Associated Neutrophils in Colorectal Cancer. *International Journal of Molecular Sciences.* 20(3), 529. doi: 10.3390/ijms20030529

56. Mogoantă, S. S., Vasile, I., Totolici, B., Neamţu, C., Streba, L., Busuioc, C., Mateescu, G. O. 2014. Colorectal cancer - clinical and morphological aspects. *Rom J morphol Embryol.* 55(1), 103–110.

57. Mohamed, S., Kaf, R. M., Ahmed, M. M., Elwan, A., Ashour, H. R., Ibrahim, A. 2019. The Prognostic Value of Cancer Stem Cell Markers (Notch1, ALDH1, and CD44) in Primary Colorectal Carcinoma. *J Gastrointest Cancer.* 50(4), 824–837. doi: 10.1007/s12029-018-0156-6

58. Molinari, C., Marisi, G., Passardi, A., Matteucci, L., de Maio, G., Ulivi, P. 2018. Heterogeneity in Colorectal Cancer: A Challenge for Personalized Medicine? *International Journal of Molecular Sciences.* 19(12), 3733. doi: 10.3390/ijms19123733

59. Mukohyama, J., Shimono, Y. A.-O., Minami, H., Kakeji, Y., Suzuki, A. 2017. Roles of microRNAs and RNA-Binding Proteins in the Regulation of Colorectal Cancer Stem Cells. LID – E143 [pii] LID – 10.3390/cancers9100143 [doi]. *Cancers (Basel).* 9(10), 2072–6694. doi: 10.3390/cancers9100143

60. Naito, Y., Saito, K., Shiiba, K., Ohuchi, A., Saigenji, K., Nagura, H., Ohtani, H. 1998. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Res.* 58(16), 3491–3494.

61. Noh, M.-G., Oh, S.-J., Ahn, E.-J., Kim, Y.-J., Jung, T.-Y., Jung, S., Moon, K.-S. 2017. Prognostic significance of E-cadherin and N-cadherin expression in Gliomas. *BMC Cancer.* 17(1), 583–583. doi: 10.1186/s12885-017-3591-z

62. Oberg, H.-H., Wesch, D., Kalyan, S., Kabelitz, D. 2019. Regulatory Interactions Between Neutrophils, Tumor Cells and T Cells. *Frontiers in immunology.* 10, 1690–1690. doi: 10.3389/fimmu.2019.01690

63. Oiwa, H., Aokage, K., Suzuki, A., Sato, K., Kuroe, T., Mimaki, S., Ishii, G. 2021. Clinicopathological, gene expression and genetic features of stage I lung adenocarcinoma with necrosis. *Lung Cancer.* 159, 74–83. doi: 10.1016/j.lungcan.2021.07.001

64. Park, J. H., Powell, A. G., Roxburgh, C. S. D., Horgan, P. G., McMillan, D. C., Edwards, J. 2016. Mismatch repair status in patients with primary operable colorectal cancer: associations with the local and systemic tumour environment. *Br J Cancer.* 114(5), 562–570. doi: 10.1038/bjc.2016.17

65. Park, J. S., Huh, J. W., Park, Y. A., Cho, Y. B., Yun, S. H., Kim, H. C., Chun, H.-K. 2015. Prognostic comparison between mucinous and nonmucinous adenocarcinoma in colorectal cancer. *Medicine.* 94(15), e658–e658. doi: 10.1097/MD.0000000000000658

66. Pérez-Carbonell, L., Ruiz-Ponte, C., Guarinos, C., Alenda, C., Payá, A., Brea, A., Jover, R. 2012. Comparison between universal molecular screening for Lynch syndrome and revised Bethesda guidelines in a large population-based cohort of patients with colorectal cancer. *Gut.* 61(6), 865. doi: 10.1136/gutjnl-2011-300041

67. Perna, C., Navarro, A., Ruz-Caracuel, I., Caniego-Casas, T., Cristóbal, E., Leskelä, S., Palacios, J. 2021. Molecular Heterogeneity of High Grade Colorectal Adenocarcinoma. *Cancers (Basel).* 13(2), 233. doi: 10.3390/cancers13020233

68. Pothuraju, R., Rachagani, S., Krishn, S. R., Chaudhary, S., Nimmakayala, R. K., Siddiqui, J. A., Batra, S. K. 2020. Molecular implications of MUC5AC-CD44 axis in colorectal cancer progression and chemoresistance. *Molecular Cancer.* 19(1), 37–37. doi: 10.1186/s12943-020-01156-y

69. Prince, M. E., Sivanandan, R., Kaczorowski, A., Wolf, G. T., Kaplan, M. J., Dalerba, P., Ailles, L. E. 2007. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci U S A.* 104(3), 973–978. doi: 10.1073/pnas.0610117104

70. Prizment, A., Vierkant, R., Smyrk, T., Tillmans, L., Lee, J., Sriramarao, P., Limburg, P. 2016. Tumor eosinophil infiltration and improved survival of colorectal cancer patients: Iowa women's health study. *Mod Pathol.* 29(5), 516–527. doi: 10.1038/modpathol.2016.42

71. Prizment, A. E., Anderson, K., Visvanathan, K., Folsom, A. R. 2011. Inverse association of eosinophil count with colorectal cancer incidence: atherosclerosis risk in communities study. *Cancer Epidemiol Biomarkers Prev.* 20(9), 1861–1864. doi: 10.1158/1055-9965.EPI-11-0360

72. Prizment, A. E., Vierkant, R. A., Smyrk, T. C., Tillmans, L. S., Lee, J. J., Sriramarao, P., Limburg, P. J. 2016. Tumor eosinophil infiltration and improved survival of colorectal cancer patients: Iowa Women's Health Study. *Mod Pathol.* 29(5), 516–527. doi: 10.1038/modpathol.2016.42

73. Ptok, H., Meyer, F., Croner, R. S., Gastinger, I., Garlipp, B. 2022. T stage-dependent lymph node and distant metastasis and the accuracy of lymph node assessment in rectal cancer. *European Surgery*. 54(2), 86–97. doi: 10.1007/s10353-021-00714-y

74. Qu, J., Jiang, Y., Liu, H., Deng, H., Yu, J., Qi, X., Li, G. 2017. Prognostic Value of E-cadherin-, CD44-, and MSH2-associated Nomograms in Patients with Stage II and III Colorectal Cancer. *Transl Oncol*. 10(2), 121–131. doi: 10.1016/j.tranon.2016.12.005

75. Raffone, A., Travaglino, A., Cerbone, M., Gencarelli, A., Mollo, A., Insabato, L., Zullo, F. 2020. Diagnostic Accuracy of Immunohistochemistry for Mismatch Repair Proteins as Surrogate of Microsatellite Instability Molecular Testing in Endometrial Cancer. *Pathol Oncol Res.* 26(3), 1417–1427. doi: 10.1007/s12253-020-00811-5

76. Rao, H., Chen, J., Li, M., Xiao, Y., Fu, J., Zeng, Y., Xie, D. 2012. Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis. *PLoS ONE*, 7(1). doi: 10.1371/journal.pone.0030806

77. Rasic, I., Radovic, S., Aksamija, G. 2016. Relationship Between Chronic Inflammation and the Stage and Histopathological Size of Colorectal Carcinoma. *Medical archives (Sarajevo, Bosnia and Herzegovina)*. 70(2), 104–107. doi: 10.5455/medarh.2016.70.104-107

78. Richards, C. H., Roxburgh, C. S. D., Anderson, J. H., McKee, R. F., Foulis, A. K., Horgan, P. G., McMillan, D. C. 2012. Prognostic value of tumour necrosis and host inflammatory responses in colorectal cancer. *British Journal of Surgery*. 99(2), 287–294. doi: 10.1002/bjs.7755

79. Rottmann, B. G., Patel, N., Ahmed, M., Deng, Y., Ciarleglio, M., Vyas, M., Zhang, X. 2021. Clinicopathological significance of neutrophil-rich colorectal carcinoma. *J Clin Pathol*, jclinpath-2021-207702. doi: 10.1136/jclinpath-2021-207702

80. Sato, K., Uehara, T., Nakajima, T., Iwaya, M., Miyagawa, Y., Watanabe, T., Ota, H. 2021. Inverse correlation between PD-L1 expression and LGR5 expression in tumor budding of stage II/III colorectal cancer. *Annals of Diagnostic Pathology*. 52, 151739. doi: https://doi.org/10.1016/j.anndiagpath.2021.151739

81. Sayar, I., Akbas, E. M., Isik, A., Gokce, A., Peker, K., Demirtas, L., Gürbüzel, M. 2015. Relationship among mismatch repair deficiency, CDX2 loss, p53 and E-cadherin in colon carcinoma and suitability of using a double panel of mismatch repair proteins by immunohistochemistry. *Polish Journal of Pathology*. 3, 246–253. doi: 10.5114/pjp.2015.54958

82. Senore, C., Basu, P., Anttila, A., Ponti, A., Tomatis, M., Vale, D. B., Segnan, N. 2019. Performance of colorectal cancer screening in the European Union Member States: data from the second European screening report. *Gut.* 68(7), 1232. doi: 10.1136/gutjnl-2018-317293

83. Shinagawa, T., Tanaka, T., Nozawa, H., Emoto, S., Murono, K., Kaneko, M., Watanabe, T. 2017. Comparison of the guidelines for colorectal cancer in Japan, the USA and Europe. *Ann Gastroenterol Surg*. 2(1), 6–12. doi: 10.1002/ags3.12047

84. Siegel, R., Miller, K., Jemal, A. 2020. Cancer statistics, 2020. *CA Cancer J Clin.* 70(1), 7–30. doi: 10.3322/caac.21590

85. Sun, X., Zhao, D., Long, S., Chen, S., Cai, Q., Yao, S. 2020. Clinicopathological and molecular features of colorectal cancer with synchronous adenoma. *Scandinavian Journal of Gastroenterology*. 55(9), 1063–1071. doi: 10.1080/00365521.2020.1795922

86. Thibaudin, M., Limagne, E., Hampe, L., Ballot, E., Truntzer, C., Ghiringhelli, F. 2022. Targeting PD-L1 and TIGIT could restore intratumoral CD8 T cell function in human colorectal cancer. *Cancer Immunology, Immunotherapy*. doi: 10.1007/s00262-022-03182-9

87. Tirumani, S. H., Shinagare, A. B., O'Neill, A. C., Nishino, M., Rosenthal, M. H., Ramaiya, N. H. 2016. Accuracy and feasibility of estimated tumour volumetry in primary gastric gastrointestinal stromal tumours: validation using semiautomated technique in 127 patients. *European radiology*. 26(1), 286–295. doi: 10.1007/s00330-015-3829-6

88. Ueno, H., Shinto, E., Shimazaki, H., Kajiwara, Y., Sueyama, T., Yamamoto, J., Hase, K. 2014. Histologic Categorization of Desmoplastic Reaction: Its Relevance to the Colorectal Cancer Microenvironment and Prognosis. *Ann Surg Oncol*. doi: 10.1245/s10434-014-4149-9

89. Van den Bossche, J., Laoui, D., Naessens, T., Smits, H. H., Hokke, C. H., Stijlemans, B., Van Ginderachter, J. A. 2015. E-cadherin expression in macrophages dampens their inflammatory responsiveness in vitro, but does not modulate M2-regulated pathologies in vivo. *Scientific reports*. 5, 12599–12599. doi: 10.1038/srep12599

90. Van Grevenstein, W. M. U., Hofland, L. J., van Rossen, M. E. E., van Koetsveld, P. M., Jeekel, J., van Eijck, C. H. J. 2007. Inflammatory Cytokines Stimulate the Adhesion of Colon Carcinoma Cells to Mesothelial Monolayers. *Digestive Diseases and Sciences.* 52(10), 2775–2783. doi: 10.1007/s10620-007-9778-4

91. Väyrynen, J. P., Tuomisto, A., Klintrup, K., Mäkelä, J., Karttunen, T. J., Mäkinen, M. J. 2013. Detailed analysis of inflammatory cell infiltration in colorectal cancer. *Br J Cancer*. 109(7), 1839–1847. doi: 10.1038/bjc.2013.508

92. Vayrynen, S. A., Vayrynen, J. P., Klintrup, K., Makela, J., Karttunen, T. J., Tuomisto, A., Makinen, M. J. 2016. Clinical impact and network of determinants of tumour necrosis in colorectal cancer. *Br J Cancer*. 114(12), 1334–1342. doi: 10.1038/bjc.2016.128

93. Vuik, F. E. R., Nieuwenburg, S. A. V., Bardou, M., Lansdorp-Vogelaar, I., Dinis-Ribeiro, M., Bento, M. J., Spaander, M. C. W. 2019. Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years. *Gut*. 68(10), 1820. doi: 10.1136/gutjnl-2018-317592

94. Vyas, M., Firat, C., Hechtman, J. F., Weiser, M. R., Yaeger, R., Vanderbilt, C., Shia, J. 2021. Discordant DNA mismatch repair protein status between synchronous or metachronous gastrointestinal carcinomas: frequency, patterns, and molecular etiologies. *Familial Cancer*. 20(3), 201–213. doi: 10.1007/s10689-020-00210-4

95. Wang, H., Wang, H. S., Zhou, B. H., Li, C. L., Zhang, F., Wang, X. F., Du, J. 2013. Epithelial-mesenchymal transition (EMT) induced by TNF-alpha requires AKT/GSK-3beta-mediated stabilization of snail in colorectal cancer. *PLoS ONE*. 8(2), e56664. doi: 10.1371/journal.pone.0056664

96. Wang, S.-M., Jiang, B., Deng, Y., Huang, S.-L., Fang, M.-Z., Wang, Y. 2019. Clinical significance of MLH1/MSH2 for stage II/III sporadic colorectal cancer. *World journal of gastrointestinal oncology*. 11(11), 1065–1080. doi: 10.4251/wjgo.v11.i11.1065

97. Wang, Z., Tang, Y., Xie, L., Huang, A., Xue, C., Gu, Z., Zong, S. 2019. The Prognostic and Clinical Value of CD44 in Colorectal Cancer: A Meta-Analysis. *Front Oncol*. 9(309). doi: 10.3389/fonc.2019.00309

98. Wei, C., Yang, C., Wang, S., Shi, D., Zhang, C., Lin, X., Xiong, B. 2019. Crosstalk between cancer cells and tumor associated macrophages is required for mesenchymal circulating tumor cell-mediated colorectal cancer metastasis. *Molecular Cancer*. 18(1), 64. doi: 10.1186/s12943-019-0976-4

99. Wikberg, M. L., Ling, A., Li, X., Oberg, A., Edin, S., Palmqvist, R. 2017. Neutrophil infiltration is a favorable prognostic factor in early stages of colon cancer. *Hum Pathol*(68), 193–202. doi: 10.1016/j.humpath.2017.08.028

100. Witschen, P. M., Chaffee, T. S., Brady, N. A.-O., Huggins, D. N., Knutson, T. A.-O., LaRue, R. S., Schwertfeger, K. L. 2020. Tumor Cell Associated Hyaluronan-CD44 Signaling Promotes Pro-Tumor Inflammation in Breast Cancer. *Cancers (Basel)*. 12(5), 1325. doi: 10.3390/cancers12051325

101. Wong, M. C. S., Huang, J., Lok, V., Wang, J., Fung, F., Ding, H., Zheng, Z.-J. 2021. Differences in Incidence and Mortality Trends of Colorectal Cancer Worldwide Based on Sex, Age, and Anatomic Location. *Clinical Gastroenterology and Hepatology*. 19(5), 955–966.e961. doi: https://doi.org/10.1016/j.cgh.2020.02.026

102. Xi, Y., Xu, P. 2021. Global colorectal cancer burden in 2020 and projections to 2040. *Translational Oncology*. 14(10), 101174. doi: https://doi.org/10.1016/j.tranon.2021.101174

103. Xu, J., Zhang, Y., Xu, J., Wang, M., Liu, G., Wang, J., Nie, G. 2019. Reversing tumor stemness via orally targeted nanoparticles achieves efficient colon cancer treatment. *Biomaterials*. 216(119247). doi: 10.1016/j.biomaterials.2019.119247

104. Yan, X., Yan, L., Liu, S., Shan, Z., Tian, Y., & Jin, Z. 2015. N-cadherin, a novel prognostic biomarker, drives malignant progression of colorectal cancer. *Mol Med Rep*. 12(2), 2999–3006. doi: 10.3892/mmr.2015.3687

105. Yan, X., Zhao, J., Zhang, R. 2017. Interleukin-37 mediates the antitumor activity in colon cancer through β-catenin suppression. *Oncotarget*. 8(30), 49064–49075. doi: 10.18632/oncotarget.17093

106. Yuan, W., Pan, Q. I., Chen, G., Yan, J., Xia, J., Chen, Y. 2015. E-cadherin expression in a rat model of acute pancreatitis. *Experimental and therapeutic medicine*. 10(6), 2088–2092. doi: 10.3892/etm.2015.2786

107. Zhang, X. F., Zhang, X. Q., Chang, Z. X., Wu, C. C., Guo, H. 2018. microRNA-145 modulates migration and invasion of bladder cancer cells by targeting N-cadherin. *Mol Med Rep*. 17(6), 8450–8456. doi: 10.3892/mmr.2018.8910

108. Zhang, Z., Xu, J., Liu, B., Chen, F., Li, J., Liu, Y., Shen, C. 2019. Ponicidin inhibits pro-inflammatory cytokine TNF-alpha-induced epithelial-mesenchymal transition and metastasis of colorectal cancer cells via suppressing the AKT/GSK-3beta/Snail pathway. *Inflammopharmacology*. 27(3), 627–638. doi: 10.1007/s10787-018-0534-5

109. Zlatian, O. M., Comanescu, M. V., Rosu, A. F., Rosu, L., Cruce, M., Gaman, A. E., Sfredel, V. 2015. Histochemical and immunohistochemical evidence of tumor heterogeneity in colorectal cancer. *Rom J morphol Embryol*. 56(1), 175–181.